STUDIES

FROM THE

PHYSIOLOGICAL LABORATORY.

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OWENS COLLEGE, MANCHESTER.

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PREFACE.

In 1877 my predecessor, DR. ARTHUR GAMGEE, *issued a volume entitled* "STUDIES FROM THE PHYSIOLOGICAL LABORATORY OF OWENS COLLEGE." *I have therefore deemed it advisable to retain the title for the present volume, which, is published by the Council of the College, and contains the results of some of the investigations carried on in the Physiological Laboratory during the last two or three years. Some of the papers are reprinted from the* JOURNAL OF PHYSIOLOGY, *from the* QUARTERLY JOURNAL OF MICROSCOPICAL SCIENCE, *and from the* JOURNAL OF ANATOMY AND PHYSIOLOGY. *These papers are reprinted with the permission of the Editors of these Journals.*

Several of the papers appear for the first time. Amongst these, Dr. Stewart's papers on "Loss of Heat by Radiation" and "The Electrolysis of Animal Tissues" are very important contributions to the subjects on which they treat.

WM. STIRLING.

PHYSIOLOGICAL LABORATORY,
THE OWENS COLLEGE,
December, 1890.

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On Red and Pale Muscles in Fishes.

BY

WILLIAM STIRLING, M.D., Sc.D.,
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For a long time anatomists and physiologists have been acquainted with the fact that the muscles of different animals differ notably in colour, structure, and physiological properties, and that even in the same animal there are differences in colour amongst the muscles. Everyone is familiar with the fact that the muscles of the breast of a fowl are lighter in colour than those of the legs; and even after being cooked this difference is quite marked, The muscles of the breast in some birds also differ in colour; thus in the partridge the pectoralis major is dark coloured, while the pectoralis minor is much lighter in tint. Similar differences obtain in the rabbit, as was shown by W. Krause* in 1868. Thus the semitendinosus is a *red* muscle, while the vastus internus and adductor magnus are *pale* muscles. That the colour of the red muscles is not due to the blood present in their blood vessels was proved by W. Kühne,† who washed out the blood by means of a half per cent solution of common salt, and found that the muscles still retained their colour—that, in fact, it was incorporated with the *sarcous* substance, and was identical with hæmoglobin, giving a spectrum the same as that of the blood pigment.

The more recent observations of MacMunn have shown that in certain muscles the colouration is due to the presence of a special pigment called by him myo-hæmatin. Ranvier‡ observed that the red and pale muscles of the rabbit differed not only in colour but also in histological and physiological properties. *Structurally* the fibres of the *red* muscles, as compared with the pale, are smaller in diameter, while the muscle-corpuscles or nuclei are numerous and somewhat spherical in shape, and placed peripherally under the sarcolemma; in the pale muscles, however, they are fusiform, fewer, and scattered throughout the sarcous substance. In the pale muscles the transverse striation is less regular than in the red. They also differ in the arrangement of their blood-vessels. In the pale muscles the arrangement of the muscular capillaries is such as obtains in ordinary striated voluntary muscles. In the red muscles, however, the capillaries between the muscular fibres are more wavy, while the transverse connecting capillaries and the veins frequently have small oval or fusiform dilatations on them like little aneurisms. Physiologically they differ also. Ranvier showed that the pale muscles were not so easily tetanised as the red ones; but he estimated the number of shocks necessary to produce tetanus at far too high a number. Kronecker and Stirling, in their investigation on the "Genesis of Tetanus,"* showed that in this Ranvier was in error, as they found that 10 stimuli per second cause a tolerably complete tetanus of the red muscles, while the pale muscles of the rabbit require 20-30 stimuli per second to produce complete tetanus. The duration of the contraction of the red muscles further is nearly twice as long as that of the pale. The red muscles therefore contract more slowly, and their contraction is more sustained and prolonged. They thus form a less highly evolved form of muscular tissue—*i.e.*, speaking physiologically as regards mere rapidity of contraction.

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* *Die Anatomie d. Kaninchens.* 1868.

† Ueber d. Farbstoff d. Muskeln. *Virch. Arch.* 1865.

‡ *Archives de Physiol. Norm., &c.* 1874, p. 5.

Meyer has described similar differences in the colour and general structure of the muscles of the guinea pig.†

A similar distinction exists also in the muscles of *Insects*, as shown by R. V. Limbeck.‡ Thus in the common water beetle, *Dytiscus marginalis*, even with the unaided eye, on opening the abdomen and thorax, one sees a sharp contrast in the colour of the muscles exposed to view. While the muscles in the abdomen are pale, the muscles which almost fill the thorax have a yellowish, indeed almost a brownish colour, and are thus sharply marked off from the others. They differ markedly in structure.

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* *Journal of Physiol.* i., p. 384.

† Ueber rothe u. blasse quergest. Muskeln, *Archiv. f. Physiol.* von du Bois Reymond, p. 217. 1875.

‡ Zur Kenntniss d. Baues d. Insectenmuskeln. *Sitzb. d. k. Akad. d. Wissensch. Math. Nat. Clas.* Bd. xci. 1885.

FISHES.—Gunther says as regards *fishes*, that 'Each lateral 'muscle is divided by a medium longitudinal groove into a 'dorsal and ventral half; the depression in its middle is filled 'by an embryonal muscular substance, which contains a large 'quantity of fat and blood-vessels, and therefore differs from 'ordinary muscle by its softer consistency, and by its colour, 'which is reddish or grayish.'*

Arrangement of Muscles.—The muscles of an osseous fish are arranged into vertical segments or *myotomes* corresponding in number with the vertebræ, and separated from one another by flat septa of connective tissue, while the fibres run in a fore and aft or horizontal course from one septum to the next one. They are therefore very short, and the connective tissue is peculiar in this respect, that boiling for a few minutes suffices to dissolve it, so that the muscular myotomes are easily separated one from another. The myotomes are connected internally with the bones of the vertebral column, and externally with the skin. The myotomes do not run in the same vertical plane, but pursue a zig-zag course. From the dorsal middle line they incline backwards then forwards, then backwards, and then bend at an angle forwards to reach the middle line, so that the apices of the parts above the middle line look backwards, and those at it look forwards, while below this line another series of apices are directed towards the tail. One set is described as a dorsal longitudinal muscle with tendinous insertions† and the other as a ventral longitudinal muscle.

Colour of the Muscles.—I daresay everyone is more or less familiar with the fact that, when a *herring* is boiled and the skin stripped off, a thin layer of reddish-brown tint is seen chiefly along the sides, and shading off dorsally and ventrally under this is the paler muscle. In the unboiled or fresh condition there is also a sharp difference in colour between these two muscles, the darker one corresponds to the red muscles of rabbits, and the deeper and far bulkier myotomes of lighter tint to the pale white muscles. The red fibres are arranged in series with the myotomes. The red muscle is thickest along the lateral line, although it does not follow this line exactly, and it shades off dorsally and ventrally. The exact distribution and thickness of this muscle is best seen in a transverse section through the body. The red muscle is crescentic in shape and thickest in the interval between the dorsal and ventral portions of the myotomes, and tapering away to become thinner as it passes dorsally and ventrally. A thin slip from it passes inwards horizontally at each myotome, to be attached to the vertebral column. Two thin red muscles diverge from the middle line just at the base of the tail, to be inserted into the fin rays.

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* *Introduction to the Study of Fishes*, p. 93.

† Owen, *Comp. Anat. and Phys. of Vertebrates*, i. p. 204.

Whiting.—A similar arrangement of a segmented lamina of a coloured or red muscle exists in the whiting, but when boiled the colour passes more into the yellow. It does not form, however, quite so continuous a layer as in the mackerel, being thickest over the centre of each myotome and thinner opposite the connective tissue septa. It is thickest just under the position of the lateral line and between the dorsal and ventral parts of the myotomes, being wedge-shaped in section. From this wedge-shaped part, fibres directed longitudinally pass down to the vertebral column. Two other thin wedge-shaped parts exist in the intervals between adjoining cones of the myotomes, one above and one below, on each side, and some prolongations of these pass inwards to reach the middle line. Some coloured fibres exist in connection with the dorsal fins.

Mackerel.—A similar red muscle exists in the mackerel, where, however, it is deeper tinted and more highly developed. It is crescentic in shape and is continued inwards on both sides as a well-marked horizontal prolongation of muscular substance attached to the vertebral column. The red muscle is so strongly marked that it at once arrests the attention when a transverse section of the body of the animal is made.

Haddock.—A similar arrangement exists in this fish. There is the corresponding radiating muscle attached to the rays of the tail. Similar red muscles exist in many fishes, *e.g.*, the salmon, and many others.

In flat fish, such as the *plaice*, the red muscle is well developed. After being boiled these muscles assume a light-brown tint. The fishes were all boiled to enable the skin to be readily removed. The red coloured muscle follows the zig-zag course of the myotomes, being thickest along the mesial lateral line, and thinner as it goes outwards; while it is thickest over the centre of each myotome, and thinner over the septa of connective tissue. A thin separate red muscle, blended with or rather lying in a depression of a white one, is to be seen along the marginal fin rays. The latter appear most distinctly when the fish is skinned and left exposed to the air for a time, when the pigment, even though altered by boiling, assumes a deeper tint. In other flat fish, such as the flounder, a similar red muscle exists.

Histological Structure.—On making a transverse section of a whiting or haddock across the mesial lateral line, so as to include the red muscle at its thickest part, and also the pale muscles, a low-power view reveals the subcutaneous fascia investing the muscle, and sending a well-marked septum to the vertebral column. Other concentric septa separate the muscular fibres into lamellæ. It will be seen at once that there are two kinds of muscular fibres—one (the red) lying under the fascia, and continued as a thinner layer inwards to the vertebral column, and the other forming the great mass of the laminæ—the pale white muscles. On transverse section the red muscle appears darker, and its sectional area is smaller than the pale fibres, which are thicker fibres altogether. In this respect they correspond to the red muscles of the rabbit. There is no difficulty in separating the groups of red fibres from the pale ones. All are supported by a certain amount of connective tissue—the septa playing the part of a perimysium, from which fibres proceed as a sustentacular endomysium.

The sharp distinction between these two kinds of muscle is far better marked in the mackerel. In a transverse section the red muscles are seen lying in a depression of the pale ones, and accurately mapped off from the latter by a septum. The red fibres are much smaller than the pale ones. Between the large pale fibres are many fat-cells, forming a nearly continuous network between the fibres, while this exists to a much less degree in the area of the red muscles. It is very probable that the condition of these fat-cells varies at different periods of the year.

If one of the red muscles of a whiting or mackerel be teased out so as to isolate a muscular fibre, on examining it with an ordinary power of the microscope, it is seen to be transversely striated, but the striæ may not be very marked, and nuclei are seen with difficulty. What is very remarkable, however, is the existence in the substance of the sarcous matter of rows of small, bright, refractive granules, in some fibres assuming a more or less longitudinal course, three, four, or more rows, being found abreast in a fibre. In others the granules may be more densely packed, and assume a more transverse direction. All the red fibres are not equally granular—some being more so, others less. The small granules are undoubtedly *fatty* in their nature, for, apart altogether from their optical characters, they are soluble in ether, and are blackened by osmic acid. When ether is added they disappear, and the fibre assumes the appearance of a transversely striated fibre. A transverse section of such fibres is particularly instructive, for we see the polygonal shape of the fibres, and note that the granules lie in the substance of the sarcous matter. It is no uncommon thing to meet with granules in the substance of muscle. In the muscles of some insects they are particularly well marked, but in these cases they are not fatty in their nature. They are spoken of as 'interstitial granules.' Flögel has described the existence of granules in the muscles of insects, and Biedermann * has shown that they occur in the fibrillæ of the thoracic muscles of the humble-bee. These granules differ both in their position in the sarcous substance and in their chemical nature, from those found in the red muscles of fishes. These fibres composing the red muscles, present all the appearances of a muscle in a state of fatty infiltration or fatty degeneration.

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* Zur Lehre v. Bau d. quergest. Muskelfaser, *Sitzb. d. k. Akad. d. Wiss. zu Wien*, lxxiv.

As to the exact position of the fatty granules in relation to the elements of a muscular fibre, there is some doubt. Most probably they exist in the cement substance, or rather the sarcoglia, between the individual fibrillæ, and it is possible that they may stand in some relation to the nutrition of the fibre itself, so that, instead of being a metabolic product in the downward direction, they may have some relation to the constructive metabolism of the fibre itself. We know, from the researches of Arnold * and L. Gerlach,† that the cement substance is the channel along which the sarcous substance seems to receive its nutriment. Sulphindigotate of soda is deposited in granules in what is regarded as the cement substance between the bundles of fibrillæ.

So far I have had no opportunity of ascertaining whether the arrangement of the blood-vessels differs in the two kinds of muscles, or whether there is any difference in their chemical composition, more especially as regards the amount of glycogen they contain. In mammals the red muscles contain more glycogen than the pale ones. These points are reserved for future research, and are not without interest, seeing that in the mammal so marked differences obtain in these respects. This communication is merely a preliminary statement of some of the results already obtained.

(Reprinted from the Fourth Annual Report of the Fishery Board for Scotland.)

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* Die Abscheidung d. indigoschwef. Natrons i. Muskelgeweb. *Virch. Arch.* 1877

† Das Verhalten d. indig. Natrons i. Knorp. lebend. Thiere. Erlangen. 1876.

On the Stimulation Effects in a Polarised Nerve during and after the Flow of the Polarising Current.

BY

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THE object of the enquiry was to investigate the electrical changes which take place in a polarised nerve when it is stimulated, both during the flow and after the opening of the polarising current, so far as these changes have not hitherto been studied, or where the study of them has been incomplete. It was thought that if the gaps in our knowledge of this subject could be filled, the search for a principle by which to explain the phenomena, whose fundamental unity it is difficult to doubt, would be made at once more easy and more safe. I have tried to obtain not only qualitative but also quantitative results, relying in the latter case rather on comparison of different experiments on the same nerve, than on observations made on different nerves at different times. I shall endeavour here, as briefly as possible, to chronicle the results and to discuss them; to connect them with each other if I can, to connect them with already established facts if that be possible; and finally to suggest an explanation of them.

I. GALVANOMETRIC OBSERVATIONS DURING THE FLOW OF THE POLARISING CURRENT, WHERE THE WHOLE INTRAPOLAR AREA IS LED OFF TO THE GALVANOMETER.

1. *Stimulating electrodes in extrapolar region.*

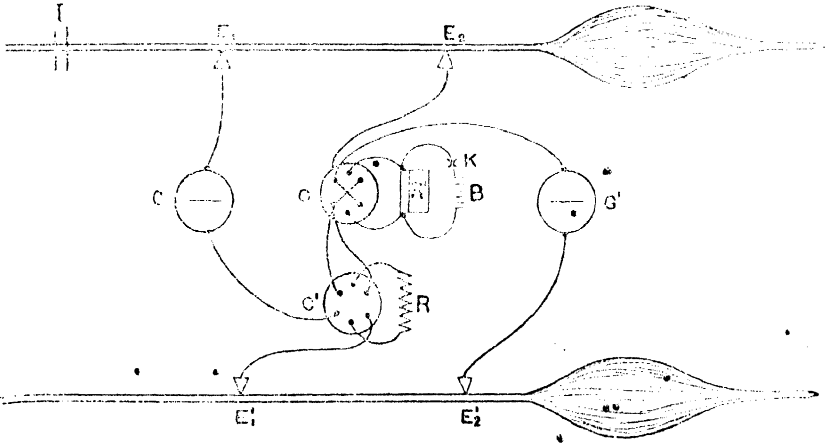
Grünhagen found in 1869 that, when a nerve is traversed by a current, the intensity of the current is apparently increased by stimulation. He explained this as due to a diminution of resistance.

Hermann, without knowing of Grünhagen's work, rediscovered the phenomenon, and investigated it more closely. He found

1. That the effect is proportional to the strength of the current only within a very limited range.
2. As the strength of the current is increased, the effect of stimulation on the side of the cathode diminishes rapidly and ultimately falls out altogether.
3. With stimulation on the side of the anode the effect goes on increasing, but not in proportion to the increase of the current.
4. A current sent transversely through the nerve is not affected by stimulation.

What I wished particularly to study was the case where stimulation is made on the anodic side. Hermann was unable to say whether in this case the effect reaches a maximum or continually increases. For the deflection became so irregular, when

FIG. 1.



I shews the position of the stimulating electrodes, which were 2 mm. apart; E_1E_2, $E'_1E'_2$ are the polarising electrodes; *G*, *G′* are the two galvanometer coils; *C* is a Pohl's commutator for changing direction of current; *C′* is a Pohl without cross wires by means of which a plug rheostat *R* could be introduced into either circuit. The circuit which did not contain *R* was completed at *C′* by a movable copper bridge.

R′ is a rheochord, *B* the battery, *K* a key.

the current was strengthened, that it was impossible to continue the observations. It was just, however, the strong currents which it was most important to study. In this difficulty a device occurred to me which has proved to be of very great service. I thought that, if the two nerves of the same frog were put one into each coil of a differential galvanometer, the current being divided between them, any irregularities in battery or nerves would be eliminated. The stimulation effect could then be got by exciting one of the nerves.

Fig. 1 shews the arrangement adopted.

As it was not possible to adjust the nerves so that the resistance should be the same in both circuits, a rheostat was connected up in such a way that it could be placed in series with either of them. Then, with a very weak polarising current, the resistance was arranged so as to bring the image to zero of the scale. The intensity of the current would then be the same in both nerve circuits, if the galvanometer constant of the two coils was the same and also the moment of the two magnets. At corresponding cross sections the current density would be approximately the same, and therefore whatever galvanometric change was caused in one nerve by the continual flow of the current would be balanced by an equal change in the other, except so far as accidental differences in the properties liable to be affected by the current are concerned. When the stronger current was now closed, the adjustment was still nearly right. The rheostat was again arranged so as to bring the image to zero, if the deflection was large. Then the stimulation effect was observed. In this way it was found possible to work with currents corresponding to an E. M. F. of 7 or 8 Daniells with as much comfort, and nearly as great accuracy, as with 1 Daniell when a "Reductionsrolle" was used.

Experiments 1 and 2 are examples of the method.

The whole rheochord contained 2,000 rheochord units.

Before adopting this arrangement I had made a series of experiments in which the irregularity was diminished by shunting the galvanometer, the deflection being balanced by a "Reductionsrolle." The results here were not at all doubtful, although the method was inconvenient and not well suited for quantitative work. Experiments 3 and 4 may serve as examples. I do not lay much stress upon the actual numbers here, except with the unshunted galvanometer; and my conclusions are based mainly upon the results of the first method.

EXPERIMENT 1.

Polarising current.		Stimulation effect.	Distance of coils of Induction machine.
1 D whole Rh	↑	+ 60	85
5 D whole Rh	↑	0	0
5 D whole Rh	↓	+ 55	0
1 D whole Rh	↓	+ 87	0
1 D whole Rh	↓	+ 55 ?	85
5 D	↓	+ 30	,,
7 D	↓	+ 14	,,
1 D whole Rh	↓	+ 85 + 90	,,

Length of nerve between electrodes 12·5 mm.

EXPERIMENT 2.

Polarising current.		Stimulation effect.	Stimulus.
1 D Rh 50	↓	+ 25	85
1 D whole Rh	↓	+ 41	,,
2 D	↓	+ 28	,,
3 D	↓	+ 17	,,
4 D	↓	+ 24	,,
5 D	↓	0	,,
1 D whole Rh	↓	+ 36	,,

Distance I E_1 20 mm.
E_1 E_2 9 mm.

EXPERIMENT 3.

Polarising current.		Stimulation effect.	Stimulus.	
1 D	↑	+ 15	200	Shunt 10
1 D	↓	+ 65	,,	,,
3·2 D	↓	+ 50	,,	,,
1 D	↓	+ 70	,,	,,
1 D	↑	+ 12	,,	,,
4 D	↓	+ 15	,,	,,
1 D	↓	+ 75	,,	,,
4 D	↓	+ 12	,,	,,
9 D	↓	+ 2	,,	,,
9 D	↓	+ 5	120	,,
1 D	↓	+ 70	200	,,

Distance between polarising electrodes 11 mm.

EXPERIMENT 4.

Polarising current.		Stimulation effect.	Stimulus.	
1 D	↓	+ 160	200	NoShunt
1 D	↑	+ 50	,,	,,
1·7 D	↑	0	,,	Shunt 10
1·7 D	↓	+ 50	,,	,,
1·7 D	↑	0	,,	,,
2·6 D	↓	+ 12	,,	,,
2·6 D	↑	0	50	,,
4 D	↓	0	200	,,
4 D	↓	+ 50	70	,,
3·2 D	↓	+ 5	200	,,
		+ 25	120	,,
2·6 D	↓	+ 4	200	,,
		+ 25	120	,,
1 D	↓	+ 5	200	,,
		+ 55	120	,,

Except when it is otherwise stated, the stimulating electrodes were at the central end of the nerve.

In all the experiments the numbers under the heading "Stimulus" refer to the distance between the coils of the induction machine expressed in millimetres. 1 D under "Polarising current" means one Daniell. The sign prefixed to the numbers in the column headed "Stimulation effect" gives the direction of the effect referred to that of the polarising stream, a + effect being one in the same direction, a – effect one in the opposite direction. In Experiments 3 and 4 the numbers representing the stimulation effect must be multiplied by the shunt number, in order to get the absolute deflection. In Experiments 1 and 2 the numbers must be doubled in order to compare with observations where the whole galvanometer was in circuit with one nerve, because here the stimulated nerve was connected up with only one coil. Again in 1 and 2 Helmholtz's wire was used, and there were two Daniells in the primary. In 3 and 4 there was only one Daniell in the primary, but the ordinary tetanising arrangement was employed. Even if the external conditions were made the same, it would not be so safe to compare the results from different nerves as to compare those from the same nerve. What is very evident from all the experiments (of course those quoted are only a sample) is that *with stimulation on the anodic side a limiting intensity of current can be reached for which the stimulation effect disappears altogether.* This lies much higher than the limiting intensity for stimulation on the cathodic side. It is raised by strengthening the stimulus in both cases, but this is much more marked for anodic than for cathodic stimulation. The longer the piece of nerve between the polarising electrodes, the greater, in general, must the E. M. F. be with which the limit is reached; in other words, the current density is the function of the current which is chiefly concerned. Experiment 3 shows very clearly that the decline of the effect as the current is increased is not due to permanent injury to the nerve, for with 1 D ↓ the effect is rather greater at the end than at the beginning. That the decline is not due to the deepening of anelectrotonus at the stimulated point, we shall be able to shew later on, when we come to speak of the comparison of the extra- and intrapolar effects. To eliminate this possibility, as far as might be, the stimulating electrodes were always placed at as great a distance from the polarising circuit as the conditions of the experiment allowed.

The curve of the stimulation effect, then, rises from a zero value and sinks to a zero value again. Between the two points where it cuts the abscissa axis it must have at least one maximum. I tried to determine the current density corresponding to which the anodic curve reaches its maximum; and it came out that this current density cannot lie very far from that for which the cathodic curve cuts the abscissa. It will be seen that in the experiments already cited the highest values of the stimulation effect for the descending current always correspond to zero, or something very like zero, for the ascending current. Thus in Experiment 3, 1 D ↓ gives the largest stimulation effect; 1 D ↑ gives one which is so much smaller that a slight increase in the current would have brought us to the zero. In Experiment 4, 1·7 D ↓ corresponds to the maximum and 1·7 D ↑ to the minimum. Experiment 1 was not directed to this point, and there is too great a gap between 1 D and 5 D. What we see is that with 1 D whole Rh ↑ the cathodic curve is already lower than the anodic (leaving out of account the doubtful observation), which is higher than with any of the stronger currents.

EXPERIMENT 5.		EXPERIMENT 6.		EXPERIMENT 7.		
Polarising current.	Stimulation effect.	E.M.F. Polarising current.	Stimulation effect.	Polarising current.	Stimulation effect.	
1/9 D ↓	+ 20	·075 D ↓	+ 15	1/25 D ↓	+ 50	
↑	+ 18	↑	+ 16	↑	+ 20	
1 D ↓	+ 130	·25 D ↓	+ 35	8 D ↓	0	even with strongest stimulation.
↑	+ 46	↑	+ 36			
2 D ↓	+ 130	·575 D ↓	+ 83	8 D ↑	0	
↑	0	↑	+ 52			
2 D ↓	+ 117	·75 D ↓	+ 99			
↑	0	↑	+ 57			
		1 D ↓	+ 97			
		↑	+ 64			

Experiment 5 is an example of observations specially devoted to this point. Experiment 6 compares the effect with the two directions for currents which were too weak to produce a complete block at the cathode.

Experiment 7 shows that in a perfectly fresh nerve the limit was certainly not higher than 8 Daniells for the descending current.

The results of this section may be summarised as follows:—

1. When stimulation is made on the side of the anode, the absolute amount of the effect increases to a certain extent with increase of current; then reaches a maximum, diminishes, and finally passes through a null value.

2. The intensity of current which corresponds to the maximum of the curve for stimulation on the side of the anode lies near that which corresponds to the minimum (null value) of the curve for stimulation on the side of the cathode.

3. Neither of the curves has a negative course below the abscissa axis for any intensity of stimulation used by me.

Both curves are near their origin straight lines. This part of the anodic curve is longer than the corresponding part of the cathodic (See Figs. 11-13).

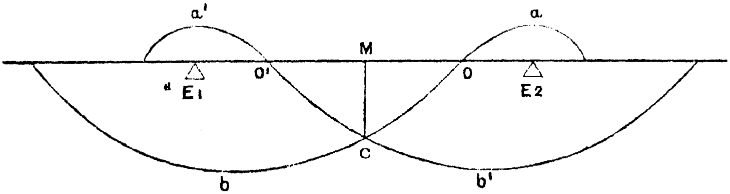
2. *Stimulating electrodes in intrapolar region.*

We have seen that there is a limit beyond which extrapolar stimulation on the side of the anode evokes no response in the intrapolar area, any more than stimulation on the side of the cathode. The block at the cathode we know to be a polar effect, because stimulation on the side of the anode is as effective as before. Besides, there is plenty of direct proof that the intrapolar area as a whole has not lost its excitability and conductivity, when the cathode has become impassable. The question, therefore, arises, Is the final disappearance of the effect due to a polar anodic block, or is it due to a depression of conductivity in the whole intrapolar region, extending also into the nearest extrapolar parts? If the depression is limited to the region immediately around the anode, there is no reason why the intrapolar effect should not persist with intrapolar stimulation long after it has ceased with extrapolar. This assumes that excitation of the intrapolar area generally is the essential condition of the stimulation effect. If the essential condition be a difference in the intensity of excitation at the two poles, in other words if polarisation increment be the main factor, then indeed intrapolar stimulation might be effective after the block was established at both poles, and yet there might be no change indicated by a galvanometer connected up with the whole intrapolar area in the usual way. We shall see later on that there is evidence to shew that the anodic block is not essentially a polar effect, as the cathodic block is, but that it is really a part of the general intrapolar depression. The very gradual decline of the effect is already a hint of this.

Arrangements. It was necessary to introduce a large resistance into the secondary coil, so as to prevent the polarising current from being short-circuited through the stimulating electrodes. A plumbago resistance of 578,000 ohms (resistance B) served this purpose effectually. Stimulation was made by closing the primary circuit, the du Bois Key in the secondary remaining open. This was to avoid any error due to the slight alteration in the total resistance of the nerve circuit which would have been caused by throwing in the secondary coil. As a rule it was not found necessary to introduce extra resistance into the nerve circuit. When it was thought necessary, a plumbago resistance of about 68,000 ohms, which will be referred to as A, was introduced. Even when there was no extra resistance in the nerve circuit, fairly strong shocks could be employed without affecting the galvanometer directly. Helmholtz's modification was used as before. The differential galvanometer arrangement was that adopted. It is unnecessary to give a figure, as everything was precisely the same as in Fig. 1, except that the stimulating electrodes were intrapolar. Except where it is otherwise stated, they were as nearly as possible in the middle of the intrapolar region. For what I wished to determine was the relative influence of the two poles, and not the absolute effect of either. It was, therefore, possible to neglect the difference of *excitability* at the point stimulated, between the unpolarised and the polarised nerve. It was only necessary that the excitability should remain the same for both directions of the polarising current. The problem was to find a point within the intrapolar region where the excitability would remain the same with the same strength of current, whether that current was ascending or descending. If one could fix the indifferent point for the two directions, then it would suffice to stimulate there. But to fix the indifferent point for each direction of every current would be almost a Herculean task, and, indeed, for the strong currents which I wished to use, it would be impossible. There is one point, however, of the intrapolar area which fulfils our condition, and that is the middle point.

For let the curve of altered excitability be drawn as in Fig. 2 for any given current. Let E_1, E_2 be the anode and cathode respectively, O, O' the indifferent points for the two directions of the current, and M the point of bisection of E_1E_2. From M draw a line perpendicular to E_1E_2 to cut the curve $aOcb$ in c. Now rotate the curve round Mc as an axis through two right angles, Mc being kept fixed in the plane of the paper. It is

Fig. 2.



clear that the new position of the curve will be $a'O'cb'$. O' will be a point on it because MO' is equal to MO. On Pflüger's theory if $aOcb$ represents the curve of altered excitability for the ascending current, $a'O'cb'$ will represent it for the descending current, and, the ordinate corresponding to M being unaltered, the excitability at M must be the same for both directions. This proof is rigorously true if the stretch of nerve E_1E_2 be homogeneous throughout in regard to those conditions which affect excitability. It would not be rigorously true, if the piece of nerve varied in its diameter, and therefore the polarising current in its density, nor if the unpolarised nerve varied in its excitability. For our experiments the excitability would be approximately the same at *M* for both directions of the current.

EXPERIMENT 8.		EXPERIMENT 9.		
Polarising current.	Stimulation effect.	Polarising current.	Stimulation effect.	
1 D Rh 100 ↑	+ 26	1 D Rh 15 ↓	+ 23	Distance between polarising electrodes 14 mm. Cross section of nerve between electrodes nearly uniform.
1 D whole Rh ↑	+ 48	↑	+ 30	
1 D ↑	+ 60	1 D Rh 100 ↓	+ 49	
2 D ↑	+ 23	↑	+ 44	
3 D ↑	+ 21	1 D ↓	+ 71	
4 D ↑	+ 13	1 D ↑	+ 69	
5 D ↑	+ 5	1 D Rh 15 ↓	+ 20	

EXPERIMENT 10. Here two pairs of nerves were used. The central end of one nerve was put in contact with the peripheral end of the other so as to equalise the cross section. The direction of the current is reckoned with reference to the nerve whose central end is next electrode E_1.

Polarising current.	Stimulation effect.	
1 D whole Rh ↑	+ 78	Stimulus 25 ; 2 D in primary ; distance between polarising electrodes, 17 mm.
↓	+ 71	
↑	+ 86	
↓	+ 78	
2 D ↓	+ 50	
↑	+ 55	
3 D ↑	+ 14	
↓	+ 15	
4 D ↓	+ 16	

These experiments show very much the same state of things as when stimulation is extrapolar and above the anode. The effect rises at first to a maximum, and then gradually declines to zero. In Experiment 8, 5 D ↑ gives very nearly the limiting current density. The maximum lies about 1 D, certainly much below 2 D. There is no hint of an indefinite persistence of the effect beyond the current density which limits it for extrapolar stimulation.

When we come to the comparison of the extra- and intrapolar effect with intrapolar stimulation, we shall find additional evidence to shew that the anode becomes impassable about the time when the intrapolar area in general has lost its conductivity and excitability. On the other hand it is seen that, long after the cathodic block has developed itself, intrapolar stimulation is effective, producing indeed something like its maximum effect with the current density which corresponds to the beginning of the block.

II. GALVANOMETRIC OBSERVATIONS DURING THE FLOW OF THE POLARISING CURRENT WHEN AN EXTRAPOLAR AREA IS LED OFF TO THE GALVANOMETER.

1. *Stimulating electrodes extrapolar.*

Let $E_1 E_2$ (Fig. 3) be a piece of nerve interposed in the galvanometer circuit, and $E_3 E_4$ in the battery circuit. Then, as has long been known, on closing the battery circuit, one obtains a current in the galvanometer circuit, the direction of which in the nerve is the same as that of the polarising current. If, now, stimulation be made, say at I, this current undergoes a negative variation.

Since Bernstein first discovered the electrotonic variation, the subject has been more than once investigated, by Hermann among others. Hermann explained the diminution which takes place alike in the anodic and cathodic currents when the nerve is stimulated, by an application of his law of polarisation increment.[1] He assumed that the excitation in passing along a polarised nerve undergoes changes in its intensity, increasing as it passes through regions under the influence of the anode, decreasing as it passes through parts dominated by the cathode.

Now, if the current be ascending in the nerve (Fig. 3), the electrotonic current in $E_1 E_2$ is also ascending. As E_2 is nearer the cathode than E_1, the excitation will pass E_2 in less intensity than E_1. Accordingly, during tetanus, E_2 may be considered as less negative than E_1. In other words, E_2 will be positive to E_1, and a current of action will pass through the galvanometer from E_2 to E_1.[1] This will have the opposite direction to the electrotonic current, and will therefore look like a diminution or negative variation of that current. Similarly if E_2 be the anode, and the current be descending, the excitation will pass over E_2 in greater intensity than over E_1, and again there will be a negative variation of the electrotonic current. Hermann, as already stated, seemed at one time to suppose that this was a complete explanation of the phenomena. But he was afterwards led by rheotome researches to modify his view, and, while retaining the law of "polarisation increment" as an essential factor in the explanation, to postulate besides, as Bernstein had previously done in a somewhat different form, an actual diminution in the polarisation, a negative variation, so to say, in the capability of the nerve to take on polarisation between core and sheath.[2]

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[1] Pflüger's *Archiv*, Bd. VI., and X.

According to Hermann, the electrotonic currents are branches of the polarising stream which spread beyond the electrodes, owing to the transverse resistance caused by polarisation between this hypothetical core and sheath. The greater the polarisation coefficient is, the more widely do these branches spread, the stronger are the electrotonic currents. If stimulation diminishes this polarisation coefficient it will, in general, diminish the electrotonic currents. If the excitation be confined to special parts of the nerve, then it will depend upon the ratio of the transition resistance between core and sheath to the longitudinal resistance of the nerve, and upon the extent and position of the unexcited or relatively unexcited parts, whether the electrotonic variation (as we may for shortness call the variation of the electrotonic currents produced by stimulation) will be negative or positive. All this he deduces from his theory, and supports by experiments with the "Kernleiter Modell." He looked in vain, however, for a positive phase in his rheotome work on nerve, the experimental difficulties being very great.

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[1] Strictly speaking, if $E_{(A)}$, $E_{(B)}$, represent the intensity of excitation at E_1 and E_2, $\int E_{(A)}dt$ is $> \int E_{(B)}dt$ for corresponding limits. Considering time-integrals, E_2 may, therefore, be looked on as positive to E_1 during the tetanus. The galvanometer deflection produced by stimulation will be a measure of the difference of these integrals.

[2] Pflüger's *Archiv*, Bd. XLII. p. 246, &c.

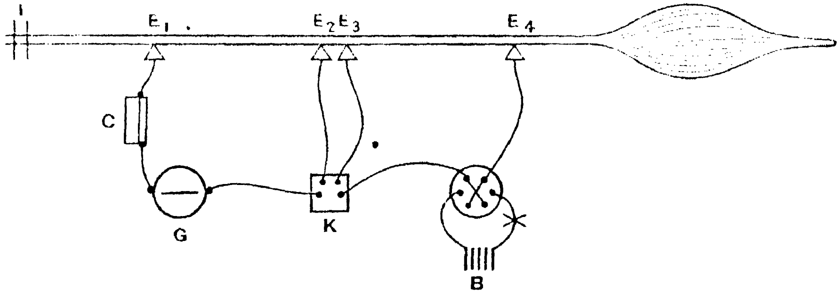
It was not from Hermann's theoretical standpoint that I entered upon the work of this section. But from experiments on the effect of stimulation on the intrapolar current during the flow, and on both extra- and intrapolar currents after the opening of the polarising stream, I suspected that, if one of the galvanometer electrodes were placed very near the polarising circuit, and the strength of the current increased sufficiently, a positive electrotonic variation ought to appear on the side of the anode, but not on that of the cathode. For the explanation of those experiments it was assumed, and the assumption was supported by direct experiments on muscular contraction, to be mentioned further on, that during the flow of the polarising current the conductivity of the nerve for the excitatory change is less around the cathode than around the anode, and that, with increasing strength of current, complete block occurs sooner at the former than at the latter, although eventually it prevails at both.

Going back now to Fig. 3, let us inquire what the effect would be on the side of the anode, *i.e*, with descending current, at a time when complete block was established there, and at the same time let us suppose that the galvanometer circuit is brought quite close to the anode, so that the lower galvanometer electrode is within the non-conducting region. If stimulation be now made at I, the excitation will pass E_1 with a certain intensity, but will altogether fail before reaching E_2. E_2 will, therefore, be strongly positive to E_1. We leave out of account for the moment any possible effect of the excitation on the electrotonic currents as such. There will be a current of action developed in the descending direction through the nerve—that is, in the same direction as the anodic electrotonic current. If this true action-current be not masked by an overwhelming negative electrotonic variation, it will appear as a positive variation of the electrotonic current.

Now let us take the case of the ascending current in Fig. 3. Here the lower galvanometer electrode is in the cathodic region, and we know that even with comparatively weak currents the cathodic block appears. E_2 will therefore, above a low limit of current density, be positive to E_1 when the nerve is excited, and the true action stream will be descending. The cathodic electrotonic current, however, is ascending, and the action stream will appear as a negative variation of it.

The results of my experiments on the intrapolar effect made me anxious to use stronger currents than Hermann had used, and to investigate the direction of the extrapolar effect especially on the side of the anode. For it was to be expected that, as soon as the anodic block was established, a resultant action current[3] must be caused between any extrapolar point and the anode itself, or a point near the anode. This current must have the opposite direction to the negative electrotonic variation, and, if it were large enough to overcome the latter, it ought to appear as a positive variation. On the side of the cathode, however, it would have the same direction as the electrotonic variation,

FIG. 3.



E_1 E_2 are the galvanometer electrodes ; E_3 E_4 polarising electrodes.
G is the Galvanometer. C the Compensator. B the battery.
K a double key by which battery and galvanometer circuits were closed simultaneously.

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[3] By resultant action current is meant the algebraic sum of all the action currents set up in a given interval. The galvanometer would practically carry out the integration.

and would only tend to strengthen this. I kept also the possibility in view that the true electrotonic variation itself, whatever its cause, might not necessarily have the same direction with strong currents which rendered the intrapolar region non-conducting, as with weak.

Since it was to be expected that the extrapolar parts of the nerve in the immediate neighbourhood of the anode would have their conductivity most reduced, the indication was to approach the lower leading-off electrode as near as possible to the upper of the polarising pair. The electrotonic currents, which were very large, were carefully compensated in order to eliminate the ordinary positive variation, or "change of resistance" effect, which could not otherwise be taken account of. To make sure that this compensation did not essentially alter the conditions determining the spread of the electrotonic currents, a separate set of observations was taken where there was no compensation. Fig. 3 shews the arrangement at first used, details being omitted.

EXPERIMENT 11.

Polarising current.	Stimulation effect.	
1 D ↓	− 60	distance of E_2 from E_3 1½ mm.
5 D ↓	+ 125	,,
5 D ↑	− 58	,,
5 D ↓	− 12	distance of E_2 from E_3 8 mm.
5 D ↑	− 19	

Stimulus, Helmholtz wire, distance of coils 85.

Distance between I and E_1 8 mm.
,, ,, E_1 and E_2 9 mm.
,, ,, E_3 and E_4 9 mm.

EXPERIMENT 12.

Polarising current.	Stimulation effect.	
1 D ↓	− 26	
2 D ↓	+ 25	current had been 4′ closed.
3 D ↓	+ 62	
4 D ↓	+ 135	
5 D ↓	+ 138	
7 D ↓	+ 126	
10 D ↓	+ 106	
4 D ↓	+ 115	

Stimulus 85; I E_1 9 mm.
E_1 E_2 9 ,,
E_2 E_3 1½ ,,
E_3 E_4 9 ,,

EXPERIMENT 13.

Polarising current.	Stimulation effect.
1 D ↑	− 19
2 D ↑	− 33
3 D ↑	− 37
5 D ↑	− 45 ?
10 D ↑	− 44

The nerve was the companion nerve of that used in Experiment 12. Distances the same as in Experiment 12.

EXPERIMENT 14.

Polarising current.	Stimulation effect.	
1 D ↓	− 45	distance I E_1 8 mm.
2 D ↓	− 73	E_1 E_2 9 ,,
3 D ↓	− 68	E_2 E_3 7 ,,
1 D ↓	− 30	E_3 E_4 9 ,,
5 D ↓	− 47	
7 D ↓	− 27	
7 D ↓	− 28	without compensation.
7 D ↓	− 37	distance E_2 E_3 3 mm.
1 D ↓	− 5	
1 D ↓	− 26	distance E_2 E_3 1 mm.
3 D ↓	+ 34	
5 D ↓	+ 26	

Only one coil of galvanometer used in this experiment.

EXPERIMENT 14 a.

Polarising current.	Stimulation effect.		
1 D Rh 1000 ↑	− 85		
	− 55	45″ after opening.	
	− 36	2′ ,,	
	− 17	4′ ,,	
	− 3	6′ ,,	
1 D Rh 100 ↑	+ 6	30″ after closure.	
	− 18	in 1′ more	Here the current was allowed to flow, the stimulation effect being read at intervals.
	− 22	1′ ,,	
	− 28	1′ ,,	
	− 35	1′ ,,	
	− 38	1′ ,,	
	− 43	15″ after opening.	
2 D ↑	− 106		
	− 129	30″ after opening.	
	− 114	1′ more.	
	− 91	1′ ,,	
	− 68	2′ ,,	
	− 57	2′ ,,	
	− 53	2′ ,,	
	− 51	2′ ,,	
3 D ↑	− 95 ?		
	− 117	20″ after opening.	
	− 86	1′ more.	
	− 68	2′ ,,	
	− 62	1′ ,,	
3 D ↓	+ 235		
closed for 5′	+ 48	30″ after opening.	
	+ 2	1′ more.	
	− 17	1′ ,,	
	− 42	2′ ,,	
	− 46	6′ ,,	

Stimulus 14. Distance $I\ E_1$ 11 mm.
$E_1\ E_2$ 9 ,,
$E_2\ E_3$ 0 ,, i.e. E_2 and E_3 united.
$E_3\ E_4$ 9

“After opening” means after opening the polarising current.

Experiments 15 and 16 are examples of the experiments where the electrotonic currents were not compensated.

EXPERIMENT 14 b.

Polarising current.	Stimulation effect.	
1 D ↑	− 98	
	− 47	30″ after opening.
	− 42	1′ ,,
	− 31	2′ ,,
	− 21	4′ ,,
	− 17	6′ ,,
1 D ↑	−112	
	−118	15″ after opening.
1 D ↑	−127	
	− 97	20″ after opening.
	− 53	5′ ,,
	− 51	6′ ,,
2 D ↑	− 67	
	− 74	30″ after opening.
2 D ↑	− 62	
	− 65	25″ after opening.
	− 55	7′ ,,

Stimulus 14. Distance $I\ E_1$ 12 mm.
$E_1\ E_2$ 11 ,,
$E_2\ E_3$ 0 ,,
$E_3\ E_4$ 9 ,,

EXPERIMENT 14 c.

Polarising current.	Stimulation effect.	
1 D 200 Rh ↑	−24	
	−39	10″ after opening.
	−28	1′ ,,
	−20	2′ ,,
	−10	7′ ,,
1 D Rh 100 ↑	−18	
	−13	20″ after opening.
	0	2′ ,,
1 D Rh 50 ↑	−10	
	− 4	15″ after opening.
	0	1′ ,,
1 D ↑	−58	
	−65	35″ after opening.
	−34	in 2′ more.

Stimulus 14. Distance $I\ E_1$ 11 mm.
$E_1\ E_2$ 9 ,,
$E_2\ E_3$ 0 ,,
$E_3\ E_4$ 9 ,,

EXPERIMENT 15.

Polarising current.	Stimulation effect.
1 D Rh 100 ↓	− 3
↑	− 3
1 D whole Rh ↓	−25
↑	−20
1 D ↓	−20
↑	−12
1 D ↓	−15
3 D ↑	−55
3 D ↓	+50
5 D ↑	−45
5 D ↓	+53

Distance $I\ E_1$ 9 mm,
$E_1\ E_2$ 11 ,,
$E_2\ E_3$ 2 ,,
$E_3\ E_4$ 9 ,,
Galvanometer Shunt 10.
Helmholtz wire not used.
Stimulus much stronger than in Experiments 11—14.

EXPERIMENT 16.

Polarising current.	Stimulation effect.	
1 D Rh 10 ↑	−28	
↓	−55	
1 D Rh 100 ↓	−17	Shunt 10
↑	− 3	,,
1 D ↓	−15	,,
3 D ↓	+55	,,
3 D ↑	−30	,,
2 D ↓	−14	,,
3 D ↓	+65	,,
3 D ↑	−16	,,

Distance $I\ E_1$ 15 mm.
$E_1\ E_2$ 14 ,,
$E_2\ E_3$ $1\frac{1}{2}$,,
$E_3\ E_4$ 9 ,,

To get rid of the unsteadiness of the deflection, which is especially troublesome in the case of the anodic current, I had recourse again to a method of what might be called automatic compensation, where the electrotonic currents of two nerves were made to oppose each other, the balance being completed by means of a compensator. Figs. 4 and 5 are simple schemes of two ways in which this was done. In the arrangement of Fig. 4 the nerves rested each on four electrodes. The distances E_3 E_4 and E_3' E_4' were made as nearly as possible equal, and the current from the same battery was divided between the two nerves. In the arrangement of Fig. 5 the nerves lay on the same pair of battery electrodes E_3 E_4. E_3 was a movable bridge of clay, which the nerves touched just where they began to diverge. In both cases the distances E_1 E_2, E_1' E_2' were made equal. It is clear, therefore, that in the galvanometer circuit

FIG. 4.

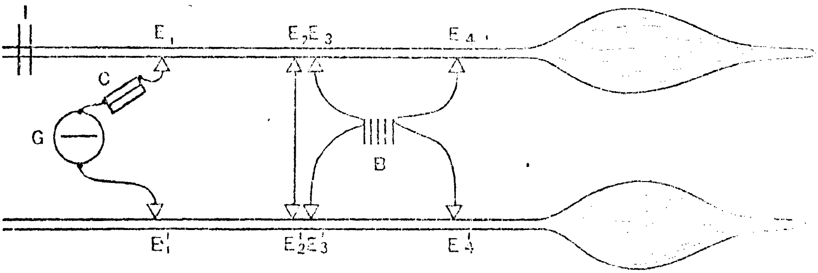
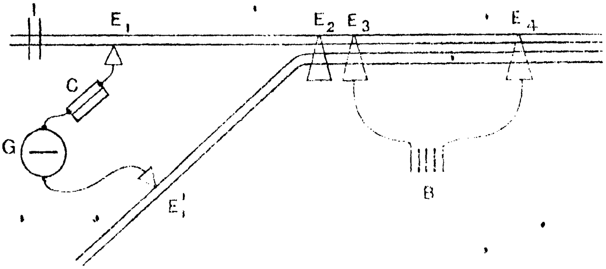


FIG. 5.



the two electrotonic currents would nearly counterbalance each other. Any irregularities due to the battery would thus be eliminated. As it was not possible to hit the balance exactly, a compensator *C* was introduced to complete it. In one set of experiments two pairs of nerves in reverse order were used, instead of two single nerves, in order to get a uniform cross section. When now one of the nerves or nerve pairs was stimulated, the stimulation effect would appear pure, but weakened by having to pass through the resistance of the included piece of unstimulated nerve. The deflection would have to be multiplied by two in order to make it comparable with the case where the galvanometer was connected only with the stimulated nerve itself. Here the resistance of the galvanometer is neglected.

I quote a few of the results got by this method, which has proved very satisfactory. Experiment 17 is an example with the arrangement of Fig. 4. Experiments 18 to 22 were done with the arrangement of Fig. 5.

Experiment 17.

Polarising current.	Stimulation effect.	
1 D ↑	− 20	
3 D ↑	− 32	
4 D ↑	− 20	
	− 19	1′ after opening.
5 D ↑	− 24	
	− 38	30″ after opening.
	− 38	1′ ”
	− 27	2′ ”
5 D ↓	+ 34	
	+ 17	30″ after opening.
	+ 9	$1\frac{1}{2}'$ ”

Distance $I\ E_1$ 9 mm.
$E_1\ E_2$ 12 ”
$E_2\ E_3$ 0 ”
$E_3\ E_4$ 12 ”

EXPERIMENT 18.

Polarising current.	Stimulation effect.	
1 D ↓	− 184	
	+ 16	20″ after opening.
3 D ↓	− 38	
	+ 46	20″ after opening.
5 D ↓	+ 58	
	+ 56	20″ after opening.
	+ 17	2′ ,,
8 D ↓	+ 68	
	+ 77	20″ after opening.
	+ 53	2′ ,,
	+ 26	6′ ,,
1 D ↓	− 138	
	+ 27	20″ after opening.
1 D Rh 100 ↓	− 53	
	+ 12	15″ after opening.
5 D ↓	+ 30	
	+ 35	20″ after opening.
8 D ↓	+ 63	
	+ 41	40″ after opening.
1 D ↓	− 79	
2 D ↓	− 76	

Distance $I\ E_1$ 10 mm.
$E_1\ E_2$ 10 ,,
$E_2\ E_3$ 2 ,,
$E_3\ E_4$ 13 ,,
Stimulus 14.

EXPERIMENT 19.

Polarising current.	Stimulation effect.	
1 D Rh 50 ↑	− 9	
1 D Rh 200 ↑	− 5	
1 D ↑	− 14	
2 D ↑	− 27	
	− 5	20″ after opening.
3 D ↑	− 36	
	− 21	20″ after opening.
5 D ↑	− 21	
(closed 4′)	− 21	20″ after opening.
5 D ↓	+ 86	
(closed 4′)	+ 24	20″ after opening.
	+ 28	1′ ,,
	+ 2	5′ ,,
5 D ↓	+ 65	
(closed 3′)	+ 22	20″ after opening.
1 D ↓	− 42	
1 D Rh 200 ↓	− 39	

Distance $I\ E_1$ 8 mm.
$E_1\ E_2$ 9 ,,
$E_2\ E_3$ 1 ,,
$E_3\ E_4$ 13 ,,
Stimulus 14.

EXPERIMENT 20.

Polarising current.	Stimulation effect.	
1 D ↓	− 33	E_2 and E_3 in contact.
3 D ↓	+ 33	
1 D ↓	− 53	distance $E_2\ E_3$ 3 mm.
3 D ↓	+ 5	
5 D ↓	+ 32	
1 D ↓	− 35	distance $E_2\ E_3$ $4\frac{1}{2}$ mm.
3 D ↓	− 24	
5 D ↓	− 19	
10 D ↓	− 1 (20″ after opening + 15)	

Distance $I\ E_1$ 8 mm.
$E_1\ E_2$ 9 ,,
$E_3\ E_4$ 12 ,,
Through whole experiment. Stimulus 14.

EXPERIMENT 21.

Polarising current.		Stimulation effect.	
1 D	↑	−16	distance E_2 E_3 4½ mm.
2 D	↑	−23	
1 D	↑	−13	E_2 E_3 3 mm.
3 D	↑	−18	
5 D	↑	−23	
1 D Rh 100	↑	− 7	E_2 and E_3 in contact.
2 D	↑	− 7	
5 D	↑	− 8 (20″ after opening −7)	
8 D	↑	− 3	
1 D	↓	−48	
3 D	↓	−93	
8 D	↓	+58	
5 D	↓	+26	

Distance same as in Experiment 20.
Stimulus 14.

EXPERIMENT 22.

Polarising current.		Stimulation effect.	
2 D	↑	−40	E_2 and E_3 very close but not touching.
1 D Rh 100	↑	− 8	
3 D	↑	−28	
5 D	↑	−23	
		−26	20″ after opening.
1 D	↑	− 9	E_2 and E_3 in contact.
2 D	↑	−14	
5 D	↑	− 3	
		−13 (20″ after opening)	
1 D	↓	−120	
2 D	↓	+ 25	
5 D	↓	+122	
		+12 (20″ after opening)	
1 D	↓	− 47	

Distance I E_1 7 mm.
E_1 E_2 7 „
E_3 E_4 11 „ Stimulus 14.

It will be seen from these results that *with currents of only moderate strength the electrotonic variation is negative both on the side of the anode and on that of the cathode, however close* E_2 *and* E_3 *may be, and even when they are united. As the current is strengthened, the negative variation of the anodic side gives place to a positive variation, and this is reached with greater difficulty the greater the distance between* E_2 *and* E_3. *On the cathodic side, however, the variation remains negative with the strongest currents I have used.* I have, indeed, in a few instances observed a small positive variation of the cathodic current, with perfectly fresh nerves and a weak polarising stream. In Experiment 14a, *e.g.*, with 1 D Rh 100 ↑, a deflection of +6 was got at first. But when the current was left closed for a longer time, this changed into a negative effect, which still increased with the time. In this connection one thinks of the fact that a weak current may establish the block at the cathode when it flows for some time, although it would not be able to do so with a shorter time of closure. The point will be referred to again.

Some observations were taken (Experiments 14, 14a, 14b, 14c, 15, 17, and 18) to compare the amount of the stimulation effect during the flow of the current with the amount a very short time after opening it. The sign of the after-effect is seen to be positive on the side of the anode, negative on the side of the cathode. This is true whether the sign of the effect during the flow on the anodic side be positive or negative.

In general it will be seen that, even 20″ after opening the polarising current, the after-effect on the cathodic side is greater than the effect during the flow, while on the side of the anode this relation is reversed. Even where this does not hold for the cathodic side, and this is almost exclusively the case for the weaker currents, the after-effect in general bears a larger proportion to that during the flow than it does on the anodic side. The meaning of this I shall try to interpret later on.

2. *Stimulating electrodes in intrapolar region.*

Here it was necessary, as before, to have the large resistance B in the circuit of the secondary coil. The stimulating electrodes, which were, of course, non-polarisable, were generally placed in the middle of the intrapolar area. Otherwise the arrangements were precisely like those for extrapolar stimulation. Experiments 23 and 24 are examples of these done according to the scheme of Fig. 3, with the stimulating electrodes intrapolar.

EXPERIMENT 23.

Polarising current.		Stimulation effect.	
1 D	↓	− 53	Stimulus 25
1 D	↑	− 8	,,
1 D Rh 16	↓	− 4	,,
1 D Rh 100	↑	0	,,
1 D Rh 400	↑	0	,,
	↓	− 32	,,
1 D	↑	0	,,
2 D	↑	− 6	,,
2 D	↓	− 15	,,
4 D	↓	0	,,
4 D	↓	− 14	Stimulus strongest
4 D	↑	− 11	,,
6 D	↑	0	,,
1 D	↓	− 63	Stimulus 25
1 D	↑	0	

Distance E_1 E_2 12 mm.
E_2 E_3 4 ,,
E_3 E_4 25 ,,
Stimulating electrodes 2 mm. apart and in middle of intrapolar region.

EXPERIMENT 24.

Polarising current.		Stimulation effect.
1 D	↑	0
1 D Rh 100	↑	− 14
1 D Rh 400	↑	− 11
1 D whole Rh	↑	0
2 D	↑	0
3 D	↑	0
3 D	↓	− 22
5 D	↑	0
1 D	↓	− 89

Distance E_1 E_2 9 mm.
E_2 E_3 8 ,,
E_3 E_4 20 ,,
Stimulating electrodes 2 mm. apart and in middle of intrapolar region.
Stimulus 25.

Experiments 25—27 were done by the method of Fig. 4, with stimulating electrodes intrapolar.

EXPERIMENT 25.

Polarising current.	Stimulation effect.	
1 D ↓	−20	Stimulus 85
2 D ↓	−83	Stimulus 10
3 D ↓	−21	”
4 D ↓	−18	”
5 D ↓	0	”
6 D ↓	0	”
3 D ↓	−49	”
4 D ↓	−24	”
5 D ↓	+ 4 (after 2′ flow; 0 at first)	”
8 D ↓	−15	”
5 D ↓	+ 3	”

Distance $E_1 E_2$ 8 mm.
$E_2 E_3$ ·3 ”
$E_3 E_4$ 24 ”
Stimulating electrodes in middle of intrapolar region.

EXPERIMENT 26.

Polarising current.	Stimulation effect.	
1 D ↓	−37	
2 D ↓	−46	
3 D ↓	−69	
4 D ↓	−12	
5 D ↓	+ 5	at first
	− 9	later
6 D ↓	− 7	
8 D ↓	0	
2 D ↓	−13	
1 D ↓	−72	
2 D ↓	−24	
4 D ↓	+ 3	
1 D ↓	−79	
6 D ↓	0	

Distance $E_1 E_2$ 12 mm.
$E_2 E_3$ 2½ ”
$E_3 E_4$ 22 ”
Stimulating electrodes in middle of intrapolar region.
Stimulus 25.

The chief points of difference between these results and those with extrapolar stimulation are (1) *the almost complete absence of any stimulation effect on the side of the cathode except with the weakest currents, and* (2) *the absence of the positive effect on the anodic side.* The first difference is without doubt due to the cathodic block. When the excitation is unable to pass the cathode the negative variation of the cathodic electrotonic current is absent, or present only in very small amount. Plainly, therefore, the important condition for this variation is stimulation of the extrapolar area itself. It is not enough that the intrapolar region, and even the extrapolar anodic region should be excited. This is of considerable interest in connection with the explanation of the electrotonic variation put forward by

Hermann, and already alluded to. It is not easy to see how a change in the polarisation constant in the intrapolar area should be unaccompanied by an effect on the cathodic electrotonic current, merely because of the block at the cathode. In the case of the intrapolar stimulation effect we know that nothing which happens in the extrapolar area has any influence, unless the excitation is able to pass the cathode. These things, on the face of them at least, seem to suggest that electrotonic variations, like other stimulation effects, only appear in parts of a nerve which are actually excited. There does not seem any reason why this should be so, if the variations are due to alteration in the polarisation between sheath and core. But it is not so easy to apply the theory to this case as it is to another, which we shall come to and of which it seems to give a ready explanation. Besides it would be absurd to lay much stress on a few experiments which only incidentally raise the question. As to the second point of difference, the absence of the positive effect on the anodic side, we see that instead of changing sign with increasing current the effect declines from its maximum to zero. This is true even when one electrode is made common to the galvanometer and battery circuits, as in Experiment 27. The positive variation is got by exciting the anodic region when the anode is impassable. It appears, therefore, for extrapolar but not for intrapolar stimulation. This we shall see more clearly from the next section.

EXPERIMENT 27.

Polarising current.	Stimulation effect.	
1 D Rh 100 ↑	- 5	Distance $E_1\ E_2$ 10 mm. $E_2\ E_3$ 0 ,, $E_3\ E_4$ 23 ,, Stimulating electrodes in middle of intrapolar region. Stimulus 25.
1 D Rh 50 ↑	- 1	
1 D Rh 100 ↓	- 8	
1 D Rh 300 ↑	+ 3	
1 D Rh 300 ↓	- 34	
1 D ↑	0	
1 D ↓	- 75	
2 D ↑	0	
2 D ↓	- 55	
2 D ↑	0	
3 D ↓	- 16	
3 D ↑	0	
5 D ↓	0	
1 D ↓	- 39	
1 D ↑	0	
1 D Rh 50 ↑	- 5	
1 D Rh 16 ↓	- 14	
1 D Rh 16 ↑	- 2	

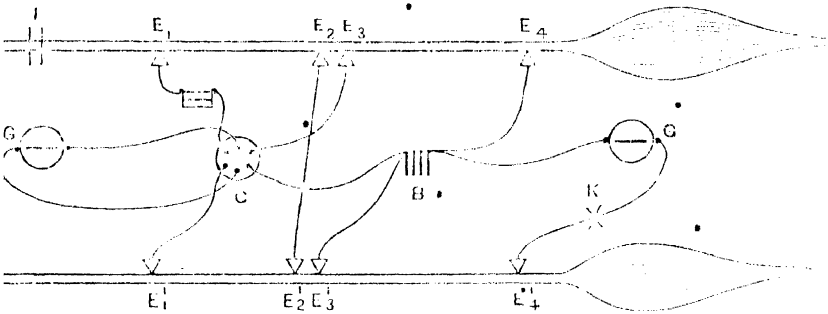
III. OBSERVATIONS DURING THE FLOW OF THE POLARISING CURRENT WHEN THE WHOLE INTRAPOLAR AREA IS LED OFF TO THE GALVANOMETER ALTERNATELY WITH AN EXTRAPOLAR AREA.

It was now desirable to compare the amount of the intra- and extrapolar stimulation effects; and in order to avoid the uncertainty which must always exist when one tries to get quantitative comparisons from experiments made on different days with different nerves, I determined to control the other observations by means of a set in which the intrapolar and extrapolar regions of the same nerve or nerve pair were led off alternately to the galvanometer. It was particularly important to notice how the *ratio* between the amount of the two effects varied with varying density of polarising current when the latter was nearly strong enough to suppress the intrapolar effect altogether.

1. *Stimulating electrodes extrapolar.*

Fig. 6 shews the arrangement generally used. By means of the commutator *C*, the second coil of the galvanometer *G*

FIG. 6.



C is a commutator by which one coil of the galvanometer *G* could be introduced into the extrapolar area E_1 E_2 or into the intrapolar area E_3 E_4. *G'* the other galvanometer coil is in circuit with the intrapolar region E_3' E_4' when the intrapolar effect is being measured, but the coil is short circuited by a du Bois key *K* when the extrapolar effect is taken.

could be put into circuit with the extrapolar or intrapolar area of the stimulated nerve at will. The other coil was only used for the intrapolar effect, the balancing arrangement being employed in order to diminish the irregularity in the deflection, which is much more troublesome than even in extrapolar experiments. Of course, only one nerve was stimulated.

In Experiment 29 the differential galvanometer method was used for the intrapolar observations, and one coil of the galvanometer for the extrapolar. The numbers would need to be multiplied by 2 in order to compare with Experiment 28 where the whole galvanometer was used. In Experiment 28 it will be seen that with 5 D ↓ the intrapolar effect is suppressed, while the extrapolar effect is not much less than with 4 D ↓. In Experiment 29 4 D ↓ is sufficient to give the limit. Put briefly, the maximum of the positive extrapolar effect on the anodic side, with extrapolar stimulation is reached with a current which does not differ much from that which gives the minimum (zero) for the intrapolar effect.

On the side of the cathode the negative extrapolar effect may apparently increase somewhat after the intrapolar effect has disappeared; but the results were a little irregular.

EXPERIMENT 28.

	Polarising current.	Stimulation effect.
Extrapolar	5 D ↓	+ 180
Intrapolar	5 D ↓	0
Fresh nerve		
Extrapolar	4 D ↓	+ 214
Intrapolar	4 D ↓	+ 50
Extrapolar	4 D ↓	+ 81
Intrapolar	4 D ↓	+ 55
Extrapolar	4 D ↓	+ 170

Distances $I\ E_1$ 9 mm.
$E_1\ E_2$ 8 „
$E_2\ E_3$ 1 „
$E_3\ E_4$ ·9 „
Stimulus 85.

EXPERIMENT 29.

	Polarising current.	Stimulation effect.
Intrapolar	4 D ↓	0
Extrapolar	4 D ↓	+ 38
	4 D ↓	+ 57
Intrapolar	4 D ↓	+ 5
Extrapolar	4 D ↓	+ 39

Here only one coil of galvanometer in circuit of stimulated nerve.

Distances and stimulus same as in Experiment 28.

2. *Stimulating electrodes intrapolar.*

Except in the position of the stimulating electrodes, the arrangement was the same as in Fig. 6.

EXPERIMENT 30.

Polarising current.	Intra-polar effect.	Extra-polar effect.
1 D whole Rh ↓	+ 137	− 37
2 D ↓	+ 55	− 21
3 D ↓	+ 12	− 5
1 D ↓		− 34
4 D ↓	+ 4	0
4 D ↓	0	
1 D whole Rh ↓	+ 131	− 44
1 D Rh 1000 ↓	+ 154	− 39
1 D Rh 500 ↓	+ 174	− 46
1 D Rh 200 ↓	+ 177	− 50
1 D Rh 100 ↓	+ 167	− 45
1 D Rh 16 ↓	+ 124	− 34

EXPERIMENT 31.

Polarising current.	Intra-polar effect.	Extra-polar effect.
unpolarised		+ 19
1 D Rh 100 ↑	+ 117	+ 5
1 D Rh 200 ↑	+ 152	+ 6
1 D 500 ↑	+ 215	+ 7
1 D Rh 1000 ↑	+ 213	+ 5
1 D whole Rh ↑	+ 291	+ 8
1 D ↑	+ 247	+ 7
2 D ↑	+ 281	+ 5
3 D ↑	+ 200	
4 D ↑	+ 44	

EXPERIMENT 32.

Polarising current.	Intrapolar effect.	Extrapolar effect.
Distance E_2 E_3 6 mm.		
1 D whole Rh ↓	+ 74	− 17
1 D ↓		− 21
2 D ↓		− 7
3 D ↓		− 5
1 D Rh 1000 ↓		− 23
1 D Rh 500 ↓		− 24
1 D Rh 100 ↓		− 16
Distance E_2 E_3 0 mm.		
1 D whole Rh ↓		− 31
1 D Rh 1000 ↓		− 27
1 D ↓		− 21
2 D ↓		− 10
4 D ↓		− 4

Distance E_1 E_2 17 mm.
E_3 E_4 25 „

In Experiments 30, 31 and 32 the stimulating electrodes were in the middle of the intrapolar area. Two pairs of nerves in reverse order were used in each experiment in order to have a uniform cross section; and in this case the direction of the current is reckoned with reference to the nerve whose central end is next E_1.

As is shown very well in Experiment 30, the intrapolar effect and the extrapolar anodic effect rise and fall together. The maxima and minima of the two effects correspond closely enough. Evidently the circumstances which favour the intrapolar, favour also the extrapolar phenomenon. This is very different from the case of extrapolar stimulation. There the extrapolar anodic variation was at its height, when the intrapolar variation had vanished, and it was positive in sign. Here there is no hint of a positive variation. Again we are forced to the conclusion that the decrease of conductivity about the anode, or in the whole intrapolar area, is the cause of the positive effect with extrapolar stimulation, the cause of the failure of all effect with intrapolar stimulation.

EXPERIMENT 33.

Polarising current.	Intrapolar effect.	Extrapolar effect.
		unpolarised 2 r
1 D Rh 16 ↑	+ 7	
1 D Rh 100 ↑	+ 29	4 r
	+ 37	
1 D whole Rh	+ 84	8 r
1 D ↑	+ 103	
		unpolarised 3 r
2 D whole Rh ↑	+ 114	7 r
3 D whole Rh ↑	+ 111	
		unpolarised 1 r
4 D whole Rh ↑	+ 63	5 r
		unpolarised 2 r
6 D whole Rh ↑	+ 28	3 r
1 D whole Rh ↓	+ 80	− 26
2 D whole Rh ↓		− 19
4 D whole Rh ↓	+ 15	− 14
6 D whole Rh ↓		− 12
9 D whole Rh ↓		0
1 D whole Rh ↓		− 22

Distance $E_1 E_2$ 12 mm.
$E_2 E_3$ 3 „
$E_3 E_4$ 26 „

2 r means two divisions of the scale to the right.
↑ in the nerve deflects to right.

When the cathodic region is led off, the stimulation effect is always insignificant. In Experiment 31 there was a positive deflection of 19 on stimulating the unpolarised nerve. This was never reversed, but only diminished. There are two ways in which the diminution may be explained. We know that the conductivity is soon diminished round the cathode; and the smaller positive deflection might be the ordinary action current weakened to correspond with the weakened excitation. But there is another explanation. The negative variation of the cathodic electrotonic current might be strong enough only to diminish the original action current, but not strong enough to annul or reverse it. In experiment 53 a small increase of the original action current seems to be got. For the weaker currents this might be explained as due to an increase in excitability at the point of stimulation. It may, however, be that the positive intrapolar effect, acting as an increase of the polarising current for the moment, does cause a small increase in the electrotonic currents, which is masked on the anodic side by the large negative variation, but appears on the side of the cathode. It would not be difficult to settle this by special experiments. For the present, however, I am obliged to leave it open.

Before we come to the experiments after opening the polarising current, it will be well to discuss briefly the results up to this point. And at the outset it should be remembered that a deflection obtained by stimulating a nerve may be the expression of more than one process, the algebraic sum, it may be, of several terms. In the above experiments we had to take account first of the ordinary negative variation. Except in special cases, the nerve was arranged so that this should be small and negligible. If the negative variation is large, it is not possible, as a rule, to reckon up the share which it takes in the compound deflection. A mere process of addition or subtraction would land us in error. For example, if we had a large negative variation in the intrapolar area, this, as well as the ordinary "change of resistance" effect, would disappear when the excitation had to pass the cathodic block. If, in these circumstances, we were to correct our zero reading by the amount of the previously determined negative variation, it is plain that one of the most important facts would be obscured, namely, the cathodic block itself. I know of no way of avoiding the difficulty, except by making the negative variation small.

In the next place there may be currents of action peculiar to the polarised nerve, which disappear with the polarisation. If at any two points of the polarised nerve which are connected with the galvanometer the excitation arrives with unequal intensity, an action current (using the words in the sense already explained) will be set up between them. In the intrapolar region, for example, when the block at the cathode is established, there will certainly be an action current in the same direction as the polarising stream, when the excitation comes in over the anode. Nothing seems more sure than that "polarisation increment," in this sense, must play a part in the intrapolar effect.

In the extrapolar region also, when one of the galvanometer electrodes is common to the polarising circuit or very close to it, there must be an action stream between the two points led off, if an alteration of the conductivity of the nerve is caused by the current, and if this alteration is chiefly confined to the intrapolar area and its immediate neighbourhood. I propose to call an action current conditioned by a modification of conductivity which is not permanent, and therefore not due to injury, a secondary action current. These currents may be small or they may be large, but they must arise, and must take part in the stimulation deflection.

Finally, a true electrotonic variation in Bernstein's and Hermann's sense may be present, and this both in the extrapolar and intrapolar regions.

There is ample evidence, as Hermann shewed long ago, that the conductivity is reduced around the cathode even with comparatively weak currents, and that the condition deepens into utter impassability when the current is increased. There is no such direct evidence of an increase of conductivity around the anode. But there is direct evidence that the conductivity is greater around the anode than around the cathode, at least for moderately strong streams, and this is all that is required to condition a positive secondary action current in the intrapolar area. So long as there is a difference in favour of the anode, it matters not whether its absolute conductivity is above or below the normal. So far then as the *direction* of the intrapolar effect is concerned, it may be entirely a secondary action current. Its quantitative relations are also explained, at least above the point where the cathodic depression begins to shew itself. For its maximum, with extrapolar stimulation on the anodic side and for intrapolar stimulation, is reached with something like the current density which gives full cathodic block. Since the conductivity around the anode is continuously diminishing after this, the difference between it and zero (the cathodic conductivity) can never be greater.

The direction of the negative extrapolar effect on the side of the cathode can be explained in the same way. But, if the negative variation on the anodic side is an action current, it is necessary to assume that the conductivity of points nearer the anode is greater than that of points farther away, that is, practically Hermann's law of polarisation increment. This is, indeed, a legitimate assumption, but, as I have said, there seems to be no direct evidence in its favour. A change of polarisation constant is, therefore, equally in court. But as the current is increased, the conductivity diminishes around the anode and in the intrapolar region generally. On any hypothesis the time will come, when an extrapolar point remote from the anode will have a greater conductivity than the anode itself or a point close to it. This is the necessary condition for a positive secondary action current in the anodic area. This, if we suppose the direction of the electrotonic variation to be unaffected, will come into conflict with the latter, and it is the difference of the two which the galvanometer will shew. When there is complete block at the anode the difference of conductivity between the two points will be as great as it can be, unless we assume that the conductivity may increase at the point which is more remote. This is the condition, then, which will determine the maximum of the positive secondary action current. But if $u=x+y$, u may be negative if x and y are both negative; or if x is positive and y negative, but y greater than x. Again u may be positive if x and y are both positive; or if x is positive and y negative, but x greater than y. In our case u, the observed deflection, is negative with the weaker currents, positive with the stronger; and we have to consider the reason of the change of sign. Whatever the explanation of the negative electrotonic variation may be, I do not think that it is mainly a secondary action current. It occurs when the led off area is far from the polarising circuit, and with weak currents. Hermann has, indeed, supposed that his law of polarisation increment was necessary to explain the difference in amount between the anodic and cathodic variations; but he does not now maintain that this is the essence of the phenomenon.

We have seen that even when the galvanometer and battery circuits touch each other by a common electrode, the variation on the side of the anode is still negative with the weaker currents. On the side of the cathode the variation is always negative, but it seems to be less when the led off area is very near the battery circuit than when it is farther away (Experiments 21 and 22). This is the opposite of what happens with the positive anodic variation. Now the secondary action current is negative on the cathodic side, and must necessarily be greater when the two circuits are close together. The idea arises that there may be a positive variation which opposes this action current, and keeps it down, although it is not strong enough to reverse it.

On the anodic side, as the pole becomes impassable, the negative variation changes into a positive variation, which is generally much larger than the cathodic effect. The secondary action current is here positive, and, if there is a positive electrotonic variation; it would add itself to this. What reason is there for supposing that a true positive electrotonic effect is developed?

On the theory that stimulation produces a change in the capacity for polarisation it is not difficult to see how an excitation which only affects the extrapolar region, and especially an excitation which fails to reach the part of this region next the polarising circuit, would cause a strengthening of the electrotonic twig[1]. Now, under the conditions of our experiments, this was nearly always the state of affairs on the side of the cathode, and the same is true of the anodic side for currents which gave the positive variation. A positive electrotonic variation is, therefore, what we might expect.

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[1] See Hermann, Pflüger's *Archiv.* Bd. XLII. *loc. cit.*

But there is some more direct evidence. The after-effect is positive on the anodic side, negative on the cathodic, that is it has the same sign as the stimulation effect during the flow. If the after-effect is due to a secondary action current, the changes of conductivity which cause such currents during the flow must be supposed to persist for some time after opening. We shall see that there is plenty of evidence that, after opening a strong current, the conductivity is depressed around both anode and cathode, if the time of closure has been more than a fraction of a second.

In the above observations the time of closure varied from twenty seconds up to several minutes, and the conditions were, therefore, favourable for the persistence of the modifications present during the flow. The after-effects may be provisionally regarded as representing the secondary action currents. This will only be an approximation, because the effects fall away continuously with the time after opening; but they do not fall away very fast.

If now the positive anodic variation during the flow is the sum of a positive electrotonic variation and a secondary action current, the after-effect may be expected to be smaller than the combined effect. If the cathodic negative variation during the flow represents a negative secondary action current diminished by a positive electrotonic variation, the after-effect may be expected to be greater than their difference. On the whole this is the behaviour which has been observed, as is illustrated by Experiments 14a, 14b, 17, and 19.[1] Experiment 18, indeed, shews in one or two cases a positive after-effect a little larger than the positive variation during the flow. This may be because the conductivity was more depressed at the anode after than before the opening, as may well happen. But generally the after effect is decisively smaller. Again, the cathodic after-effect may occasionally be less than that during the flow. But this is chiefly with weak currents, and we shall see that it is only after the stronger currents that the depression of conductivity persists at the cathode. As has been pointed out, the after-effect can in any case be regarded only as an indication, in exceptional circumstances, of what happens during the flow. In order that it may indicate this aright, the conditions must be such that the state of affairs during the flow persists for some time after opening the current. In my experience a long period of flow is the most important of these conditions, but the strength of the current is also of importance. It is only for the extrapolar regions that the conditions can be realised.

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[1] The results cannot be due to difference of excitability at the point of stimulation during and after the flow. Any effect which this might have would rather be in the opposite direction.

On the whole, although I do not wish to press the point, there is a good deal of evidence in favour of the existence of the positive electrotonic variation. If we take the analogy of the negative electrotonic variation as a guide, it is not difficult to see why the secondary action current in the cathodic area should be able to master the electrotonic variation, because the latter may be expected to be much smaller than that on the anodic side.

The conclusion I come to is that *the stimulation effects during the flow of the polarising current cannot be explained entirely as secondary action currents, but are probably due to the superposition of such currents on electrotonic variations.* How the latter differ from ordinary action currents it is difficult to say. The main factor in their production, at least under the conditions of my experiments, is the excitation of the led off area itself. When this is cut off from the stimulus by a block which does not prevent the spread of electrotonus, for example the cathode of the polarising current, the stimulation effect is absent. Experiments 31 and 33 are apparently against this view. But the deflections there are very slight; and even if we admit that a small effect may be caused, without excitation of the actual area led off, this does not weaken the evidence which shews that such excitation is the chief factor. It is very suggestive that none of the stimulation effects, so far as my experience goes, exceeds in amount the maximum positive polarisation deflection in the intrapolar region, or the main anodic after-current in the extrapolar? They are very far from increasing indefinitely with increase of the polarising stream. Everything indicates that they are functions of a physiological rather than of a physical property of the nerve. They may, however, be functions of a physiological change in a physical property. This would explain why they should tend to a limit, but not why that limit should be the one indicated by processes which are probably purely physiological.

IV. OBSERVATIONS AFTER OPENING THE POLARISING CURRENT WHEN THE WHOLE INTRAPOLAR AREA IS LED OFF TO THE GALVANOMETER.

1. *Stimulating electrodes extrapolar.*

So far as I know the first systematic investigation of this subject was a paper published by me in the Journal of Physiology, Vol. IX., p. 26, &c., of which a preliminary notice was read before the Royal Society of Edinburgh in April, 1887.

It has been pointed out to me, that a year before this Mr. Gotch demonstrated to the Physiological Society in Oxford[1] "the polarising effect produced on nerve by the brief passage of an electric current through it, and the diminution in this effect caused by the active state of the tissue." This of course proves that Mr. Gotch knew of the direction of the intrapolar effect a year before my paper was published. Hermann, as I subsequently found, had previously observed traces not only of this effect, but also of a negative variation in the extrapolar regions, during some investigations on the stimulation effect during the flow.[2]

But he had only done so casually, in the intervals between his main experiments; and, although he observes that these effects are *probably* always present after polarisation, he does not seem to have gathered any notion of their real magnitude. The explanation of them he expressly declines to discuss until he has settled the true nature of the chief phenomena of his experiments, the stimulation effects during the flow namely. So late as 1883 du Bois Reymond says "Wenn der positive Polarisationsstrom während der Dauer des polarisirenden Stromes durch Tetanus des Nerven verstärkt wird, dies wahrscheinlich auch mit dem positiven Nachstrome der Fall sein wird."[1]

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[1] This *Journal*, Vol. VII., 1886 (*Proc. of Physiol. Soc.*, Mar. 20).

[2] Pflüger's *Archiv*. Bd. XLII. p. 246, 1881.

The special object of this part of the work was to ascertain if it made any difference in the amount of the stimulation effect, whether the excitation had to pass the anode or the cathode.

It is not necessary to enter into details as to the arrangements, as this has already been done in my previous papers. The polarising current having been opened, the nerve was thrown at once into circuit with the galvanometer; the polarisation deflections were read off; and, as soon as the image had become sufficiently steady, stimulation was made and the effect noted. Whenever a quantitative comparison was intended, the same interval was allowed between opening the polarising circuit and stimulation in every observation. In measuring the time of flow, I did not use the automatic arrangement, described in my first paper, for the shortest periods. It was all done by the watch and metronome. Irregularities are, of course, unavoidable, but they do not introduce any serious error, when spread over a whole series of observations; and what is lost in accuracy is more than made up for in convenience. In some of the experiments it was desirable that there should be no interval between the commencement of stimulation and the opening of the polarising current. Here two observations of the polarisation deflections were made, one while the nerve was at rest, the other while it was being tetanised. In the latter case the stimulation effect would be added algebraically to the polarisation.

Unless it is otherwise stated, the stimulating electrodes were at the central end of the nerve. Experiments 34 to 39 are examples.

We see here that the stimulation effect may fail altogether after the opening of a strong current, if the excitation has to pass the anode; while, if it has only to pass the cathode, we may have a large effect. We interpret this as meaning that the conductivity around the anode is less than that around the cathode soon after the opening of such a current. We shall see later on that this is especially true, when the time of flow is short. Even where the effect appears after descending current, it is in general less than after ascending. Occasionally after ↓ the sign of the effect is positive.

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[1] *Sitzungsb. der Kgl. Preuss. Akademie der Wissenschaften*, 1883, Bd. I.

EXPERIMENT 34.

Polarising current.	Time of flow.	Polarisation deflection.	Stimulation effect.	Very short time of flow means not more than ¼″.
7 D ↓	very short	− 70 + 85	− 30	Stimulus 100
10 D ↓	,,	− 110 + 190	0	,, 200 (this stimulus was quite effectual before)
,, ↑	,,	− 75 + out of field	− 15	Stimulus 200.
,, ↓	,,	− 150 + 195	+ 10 In 1′ 0	
,, ↑			− 35	

EXPERIMENT 35. Here the polarisation deflections were taken first while the nerve was at rest, and again while it was being tetanised.

Polarising current.	Time.	Tetanus.	Rest.	
10 D ↓	very short	− 265 + 270	− 170 + 260	Stimulus 100
10 D ↑	,,	− 100 + 310	− 95 + 426	,,
10 D ↑	,,	− 110 + 335	− 90 + 365	
10 D ↓	,,	− 370 + 270	− 190 + 275	
10 D ↑	,,	− 110 + 348	− 100 + 395	

EXPERIMENT 36.

Polarising Current.	Time.	Stimulation effect.	
10 D ↓	very short	0	Stimulus 100
10 D ↓	”	0	”
10 D ↑	”	− 15	1′ after opening polarising current
10 D ↑	”	− 25	

EXPERIMENT 37. Short time of flow means not more than ½″ nor less than ¼″.

Polarising current.	Time.	Stimulation effect.	
6 D ↓	Short	− 30	Stimulus 140
6 D ↑	”	− 185	”
6 D ↑	5″	− 18	In 2′ after opening current
6 D ↓	5″	0	Tendency to move in positive direction
		In 1′ − 30	
10 D ↓	1″	0	
		In 1′ − 5	
10 D ↑	1″	− 45	

EXPERIMENT 38.

Polarising current.	Time.	Stimulation effect.	
			Stimulus 140
12 D ↓	very short	+ 10	In 1′
		+ 2	In 2′
12 D ↑	”	− 84	In 30″
		− 45	2′
		− 25	3′
		− 18	4′
		− 10	5½′
		− 7	7′
		− 5	9′
12 D ↓	5″	+ 10	In 1′
		+ 3	2′
		+ 5	4′
		+ 6	9′

Experiment 39. Nerve was crushed over lower polarising electrode. There is now a strong ascending current in nerve, and on stimulation 180 divisions of negative variation.

Polarising current.	Time.	Stimulation effect.	
12 D ↓	short	+ 10	In 30″ after opening.
		+ 45	1½′ ,,
		+ 165	3½′ ,,
		+ 175	5′ ,,
12 D ↑	short	− 210	1′ ,,
		− 185	3′ ,,
		− 153	4′ ,,
		− 190	7′ ,,
		− 192	9′ ,,
			Repeated crushing. Negative variation now 207.
12 D ↑	short	− 201	In 30″ after opening.
		− 195	1½′ ,,
		− 198	3′ ,,
12 D ↓	short	+ 15	30″ ,,
		+ 80	1½′ ,,
		+ 205	3′ ,,
		+ 204	4′ ,,

Experiment 24 is very instructive and it will be well to discuss it briefly here leaving the others to be dealt with later on. It is plain that after the descending current the positive deflection got on stimulation represents the negative variation in the ordinary sense; and we may suppose that the excitation is blocked at the anode, and that the block gradually gives way. We know from the other experiments in this group that there is no negative effect after a strong descending current, so that we cannot suppose that the smallness of the positive deflection here in the first minute or two after opening the current is due to an opposing negative effect. The positive deflection is therefore in this case a measure of the conductivity of the nerve around the anode. And it never obtains a higher value than the original negative variation. But we might suppose that in the case of the ascending current we ought to get the original negative variation added to the negative deflection caused by stimulating the polarised nerve. The apparent negative stimulation effect ought then to be very much larger than the original negative variation. We see, however, that it is only a little larger after the first crushing, and actually somewhat smaller after the second. The reason is no doubt this. The excitation, passing the region of the cathode freely, is blocked in the uninjured nerve as it nears the anode after the opening of a strong current. It is effectually blocked; but it cannot be more effectually blocked than it is by the crushing of the injured nerve. In other words in the nerve which has been crushed over the anode the nerve impulse and the negativity which is bound up with it develop a certain strength upon the cathode, but fall away to nothing on the anode. Again in the uninjured strongly polarised nerve they develope on the cathode a certain strength not differing probably very much from that developed in the unpolarised nerve; and on the anode this also as we have seen falls away to nothing. Accordingly the difference of potential between cathode and anode will be pretty nearly the same in both cases, and therefore the deflection produced by stimulation will be nearly the same.

The results of a long series of observations may be summed up thus:

1. The stimulation effect is always in the direction of diminution of the positive polarisation.

2. When the polarising current is below a certain limit of intensity and its time of closure very short, no effect is produced by stimulation. (The ordinary negative variation is supposed to be eliminated by symmetrical position of the electrodes.)

3. With increase of strength of the polarising stream or increase of time of closure, the effect increases; and beneath a certain limit, it is indifferent whether stimulation be made on the side of the cathode or on the side of the anode.

4. Above this limit the effect is less with stimulation on the side of the anode than with stimulation on the side of the cathode. Ultimately the anodic curve reaches a null value.

5. The curve representing the effect with stimulation on the side of the cathode reaches its maximum with an intensity of current which lies near that for which the anodic curve cuts the axis.

6. If the cathodic curve cuts the axis, its null value corresponds to an intensity of current far above that corresponding to the null value of the anodic curve.

2. *Stimulating electrodes intrapolar.*

The circuit of the secondary coil was only closed after the opening of the polarising current.

Here it will be seen that, on the whole, the direction of the polarising current is without influence on the amount of the stimulation deflection. Small differences there are, but they eliminate each other; for sometimes after the ascending current the stimulation effect is a few divisions more than after the corresponding descending current, sometimes it is the opposite which happens. These small irregularities are no doubt partly due to the difficulty of regulating the time of closure, when it is short, by the metronome. There is no reason why with symmetrically placed electrodes, uniform cross section of the nerve, and median stimulation, the stimulation effect should depend on the direction of the current, unless there are differences of excitability and conductivity to begin with in different parts of the piece of nerve used. If there are such differences, if, for example, the nerve is crushed or seared over one of the polarising electrodes, then we should expect, from the previously quoted results, to find that when this electrode is the anode the stimulation effect should be less than when the other electrode is the anode. For we have seen that, at least for short closure time, it is around the anode that the conductivity is most lowered after the opening of the polarising current, and the conductivity of the crushed part of the nerve cannot, of course, be affected. Experiment 42 shews what happens here.

EXPERIMENT 40.

Polarising current.	Time of flow.	Polarisation deflections.	Stimulation effect.	
2 D ↑	5″	−125	−12	Two nerves in reverse order. Distance between polarising electrodes 26 mm. Stimulating electrodes in middle of intrapolar region.
2 D ↓	5″	−151	− 7	
5 D ↑	1″	−108 + 24	− 9	
5 D ↓	1″	−136 + 35	−10	
5 D ↑	5″	−375	−14	
5 D ↓	5″	−345	−13	
12 D ↓	short	−201 +190	−31	
12 D ↑	short	—	—	
10 D ↓	5″	−∞	−25	
10 D ↑	5″	−∞	−44	
7 D ↓	½″	−140 + 52	−19	
7 D ↑	½″	−102 + 68	−14	

EXPERIMENT 41.

Polarising current.	Time of flow.	Polarisation deflections.	Stimulation effect.	
5 D ↓	½″	− 70 + 63	− 7	Only part of nerve below branches for thigh used. Stimulating electrodes in middle of intrapolar region. Distance between polarising electrodes 16 mm.
5 D ↓	½″	− 77 + 32	− 5	
8 D ↑	½″	−111 + 92	−15	
8 D ↓	½″	−102 +140	−26	
12 D ↓	½″	−137 +233	−31	
12 D ↑	½″	−140 +305	−35	
12 D ↓	½″	−137 +272	−37	
5 D ↑	15″	−∞	− 9	
5 D ↓	15″	−∞	−14	
5 D ↑	15″	−∞	−12	
5 D ↓	15″	−∞	−10	

EXPERIMENT 42. Nerve crushed over lower polarising electrode.

Polarising current.	Time of flow.	Polarisation deflection.	Stimulation effect.
18 D ↓	very short	−255 +240	−19
18 D ↑	"	−197 +202	− 7
18 D ↓	"	−152 +249	−17
18 D ↑	"	−189 +170	−·8

The stimulation effect, it will be observed, is rather more than twice as great with ↓ as with ↑; that is to say, it is important that the anode should be on an uninjured part, if a large effect is to be got. A short time of flow was used here, because with a long time of flow we know that the conductivity around the cathode remains depressed, and therefore the difference could hardly be expected to be so great.

V. OBSERVATIONS AFTER OPENING THE POLARISING CURRENT, WHERE AN EXTRAPOLAR REGION IS LED OFF TO THE GALVANOMETER.

1. *Stimulating electrodes extrapolar.*

The stimulating electrodes were as usual at the central end of the nerve. In Experiments 43—49 there was an electrode common to the galvanometer and polarising circuits. The sign of the stimulation effect is reckoned as before with reference to the direction of the polarising current in the nerve.

When the polarising current is ascending, we get a cathodic after-current in the same direction; when the polarising current is descending, the main anodic after-current in the opposite direction is the one which affects the galvanometer. (The preliminary kick in the direction of the polarising current, which Hermann has investigated, was seen; but it is very transitory.)

EXPERIMENT 43.

Polarising current.	Time of flow.	Stimulation deflection.	
6 D ↓	very short	+ 25	Stimulus 200.
6 D ↑	”	− 10	”
6 D ↓	”	+ 17	”
6 D ↑	”	0	”
6 D ↓	”	+ 23	”
12 D ↑	”	− 5	”
12 D ↓	”	+ 6	”
		+ 38	Stimulus 140.
		+ 8	In 2′.
12 D ↑	very short	− 11	Stimulus 140.
12 D ↓	”	+ 16	”

EXPERIMENT 44.

Polarising current.	Time of flow.	Tetanus.	Rest.	
6 D ↓	very short	− 186		
6 D ↓	”		+ 7 − 201	
6 D ↑	”	+ 15	+ 12	
6 D ↑	1″	+ 40	+ 28	
6 D ↓		+ 30 − 150	+ 10 − 180	
				Stimulation in 30″ gives + 25.
6 D ↓		+ 40		Stimulus 140.
6 D ↑		0		”
6 D ↓	1′	+ 55		”

EXPERIMENT 45.

Polarising current.	Time of flow.	Stimulation deflection.	
6 D ↑	2″	+ 10	Stimulus 140.
6 D ↓	2″	+ 55	”
		+ 20	In 1
6 D ↑		0	
6 D ↓	30″	+ 37	
		+ 35	In 1′.
		0	In 3′.
6 D ↓	10″	+ 72	
”		+ 7	In 2′.

EXPERIMENT 46.

Polarising current.	Time of flow.	Stimulation deflection.	
6 D ↓	10″	+ 48	Stimulus 140.
1 D ↓	30″	+ 4	,,
1 D ↑	30″	+ 3	,,
3 D ↓	30″	+ 7	,,
6 D ↑	30″	− 15	,,

EXPERIMENT 47. Stimulation at *distal* end of nerve. Nerve divided from muscle.

Polarising current.	Time of flow.	Stimulation deflection.	
6 D ↑	1″	+ 30	Stimulus 140.
6 D ↓	1″	− 11	,,
6 D ↑	10″	+ 20	,,
6 D ↓	10″	− 20	,,

After the descending current, except in Experiment 47, the stimulation deflection is always in the same direction as the polarising current, or in the opposite direction to the main after-current. After the ascending stream the effect is, in the first place, in general far smaller than after the descending; secondly, it is usually, with short time of flow, of negative sign with respect to the direction of the polarising current, *i.e.*, in the opposite direction to the cathodic after-current. With longer time of flow, even with comparatively weak streams, it becomes positive and therefore has the same direction as the cathodic after-current. With a pretty strong stream, and a closure of several seconds, the positive effect after the ascending current may assume such proportions as to be of the same order as the effect after the descending current. Experiment 48 illustrates this.

Experiment 47 shews that it is not a difference of ascending and descending current with which we have to deal. For, when the stimulating electrodes are placed at the distal end of the nerve, everything is reversed. It is now after the ascending current that the large positive effect is got; after the descending current the effect is negative.

EXPERIMENT 48.

Polarising current.	Time of flow.	Stimulation deflection.		
1 D ↓	30″	− 3		Stimulus 400.
3 D ↓	30″	+ 21		”
6 D ↓	30″	0		”
		+ 7	In 1′.	”
		+ 45	In 2′.	Stimulus 140.
6 D ↓	5″	+ 90		”
6 D ↑	5″	− 25		”
6 D ↓	5″	+ 80		”
6 D ↑	5″	− 34		”
6 D ↓	5″	+ 72		”
		+ 16	In 1′.	”
		+ 4	In 3′.	”
3 D ↑	5″	+ 3		”
6 D ↑	30″	− 58		”
1 D ↓	5″	+ 2		”
1 D ↓	30″	+ 8		”
1 D ↓	3′	+ 28		”
		− 20	In 30″.	”
		− 70	In 2′.	”
		− 40	In 5′.	”
6 D ↓	10″	+ 65		”
		+ 40	In 1′.	”
		+ 30 ?	In 2′.	”
1 D ↓	3′	+ 27		”
		+ 11	In 1′.	”
		+ 2	In 2′.	”

Experiment 49 shews that with the long time of flow (2′) the effect on the cathodic side may equal that on the anodic. The great tenacity of the modification on which the effect depends is well brought out. As to the extrapolar polarisation currents, although it is usually stated that the cathodic current disappears very quickly, I am convinced that this is only true if the time of closure be short. With a time of closure of 1′ and upwards and a fairly strong polarising stream, the cathodic current seems to hold its ground even more stiffly than the anodic.

EXPERIMENT 49.

Polarising current.	Time of flow.	Polarisation deflection.	Stimulati effect.
5 D ↑	very short		− 2
5 D ↓	,,		+ 11
5 D ↓	5″	− 148	+ 19
5 D ↑	5″	+ 45	− 9
5 D ↓	5″	− 154	+ 22
5 D ↓	1′	− 182	+ 36
	in 1′ after opening		+ 25
	,, 2′ ,,	− 10	+ 26
	,, 3′ ,,		+ 18
	,, 8′ ,,		0
5 D ↑	1′	+ 162	− 28
	in 1′ after opening	+ 125	− 26
	,, 2′ ,,	+ 115	− 21
	,, 3′ ,,	+ 105	− 15
	,, 4′ ,,	+ 96	− 11
	,, 6′ ,,	+ 81	− 4
	,, 8′ ,,	+ 70	− 3
5 D ↓	2′	+ 51 − 154	+ 30
	in 1′ after opening	− 35	+ 27
	,, 2′ ,,	+ 8	+ 21
	,, 3′ ,,	+ 31	+ 15
	,, 4′ ,,	+ 52	+ 10
	,, 5′ ,,	+ 75	+ 4
	,, 7′ ,,	+ 99	− 2
	,, 9′ ,,	+ 121	− 6
	,, 11′ ,,	+ 120	− 7
	,, 15′ ,,	+ 141	− 11
5 D ↑	2′	− 245	− 31
	in 1′ after opening	− 215	− 29
	,, 2′ ,,	− 199	− 28
	,, 4′ ,,	− 188	− 23
	,, 6′ ,,	− 169	− 16
	,, 10′ ,,	− 141	− 4
	,, 12′ ,,	− 124	− 1
	,, 16′ ,,	− 103	− 4
	,, 18′ ,,	− 93	− 6
5 D ↓	1″	− 96	+ 14
5 D ↑	1″	− 30	− 5

The results of the section are as follows :—

1. On the side of the anode the stimulation effect is always in the same direction as the polarising current, or in the opposite direction to the main after-current.

2. On the side of the cathode the effect is
   - (*a*) in general far smaller than on the side of the anode.
   - (*b*) with short time of flow it is in the opposite direction to the polarising current, *i.e.*, in the opposite direction to the cathodic after-current.
   - (*c*) With longer time of flow, even with comparatively weak currents, it becomes positive in sign, *i.e.*, it has the same direction as the polarising stream.

3. With increased intensity of stream and time of flow the positive effect on the side of the cathode may assume such proportions as to be of the same order as that on the side of the anode.

2. *Stimulating electrodes intrapolar.*

Here the large resistance *B* was in the circuit of the secondary coil in order to prevent polarisation currents from passing. This circuit was open during the flow of the pol stream.

EXPERIMENT 50.

Polarising current.	Time.	Polarisation deflection.	Stimulation effect.
5 D ↓	1·3″	− 69	+ 17
5 D ↑	1·3″	+ 6	0
5 D ↓	1·3″	− 58	+ 19
5 D ↑	1·3″	+ 9	+ 2
10 D ↓	·5″	− 102	+ 41
10 D ↑	·5″	+ 31	− 14
10 D ↓	very short	− 106	+ 34
10 D ↑	,,	+ 23	− 11
5 D ↓	10″	− 126	+ 36
5 D ↑	10″	+ 40	+ 4
5 D ↓	30″	− 161	+ 46
5 D ↑	30″	+ 68	+ 2
10 D ↓	1·3″	− 95	+ 28
10 D ↑	1·3″	+ 55	− 12
10 D ↓	30″	+ 167	− 23
10 D ↑	30″	+ 342	0
	in 1½′ after opening		+ 37
	in 2½′		+ 33

Distance between polarising electrodes 17mm.
Distance between galvanometer electrodes, 9 mm.
Stimulating electrodes in middle of intrapolar region.

EXPERIMENT 51.

Polarising current.	Time.	Polarisation deflection.	Stimulation effect.
Stimulating electrodes 4 mm. from lower end of intrapolar area.			
5 D ↑	1″	+ 16	0
5 D ↓	1″	− 108	+ 39
10 D ↑	1″	+ 36	− 19
1 D ↓	1″	− 202	+ 45
Stimulating electrodes in middle of intrapolar area.			
5 D ↑	1″	+ 9	− 1
5 D ↓	1″	− 104	+ 25
10 D ↑	1″	+ 21	− 7
10 D ↓	1″	− 182	+ 36
Stimulating electrodes 3 mm. from upper end of intrapolar area.			
5 D ↑	1″	+ 7	+ 1
5 D ↓	1″	− 66	+ 19
10 D ↑	1″	+ 25	− 7
10 D ↓	1″	− 180	+ 27

Distance between polarising electrodes 22 mm.
Distance between galvanometer electrodes 9 mm.

Experiment 52.

Polarising current.	Time of flow.	Polarisation deflection.	Stimulation effect.	
		unpolarised	66	Two nerves in reverse order: crushed over upper galvanometer electrode. Stimulus 85.
10 D ↑	1″	+ 25	56	
		unpolarised	56	
5 D ↑	5″	+ 25	46	
		unpolarised	51	
5 D ↓	5″	− 118	22	
	in 3′		49	
		unpolarised	174	Stimulus increased to 50.
12 D ↓	1″	− 221	68	
	in 1′		109	
	3′		147	
	5′		155	
12 D ↑	1″	+ 41	116	
	in 1′		119	
	3′		141	
	5′		122	
12 D ↓	5″	− 360	16	
	in 1′		52	
	2′	− 72	102	
	3′		110	
	5′		143	
12 D ↑	5″	+ 134	89	
	in 1′		115	
	in 3′		123	
12 D ↑	15″	+ 280	34	
	in 45″		40	
	2′		51	
	4′		60	
	7′		67	
	12′		74	

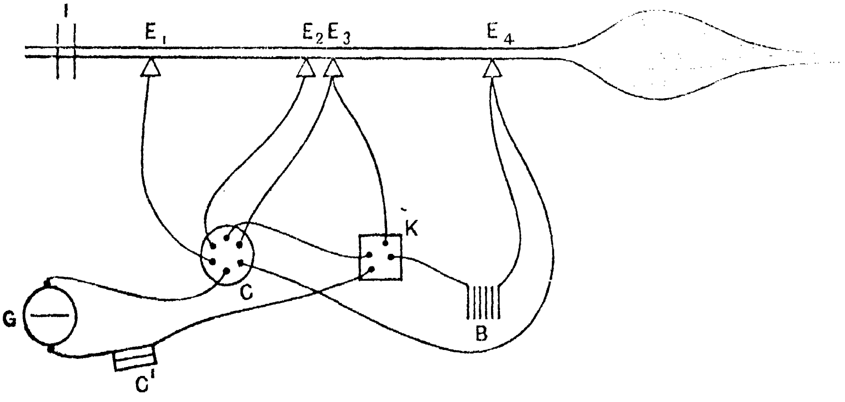
Distance between polarising electrodes 21 mm.
" " galvanometer " 12 "

Here, as before, the sign of the effect is positive in the anodic area, negative in the cathodic. Experiments 50 and 51 shew that, with the weakest currents there used and not too long a closure, there may be no effect at all on the side of the cathode. As we increase the strength of the current or the time of flow or both, the cathodic effect as well as the anodic increases, but the former goes on growing after the latter has begun again to decline, and ultimately we may have no effect at all in the anodic area immediately after opening the current, while there is a large effect on the side of the cathode. At the end of Experiment 50, for example, 10 D ↓ closed for 30″ gave no effect at first, but after an interval a large positive deflection. The absence of effect with weak currents on the cathodic side, with strong currents on the anodic side, may be due to the same cause, or to different causes. If no modification of conductivity be left around either pole, then on our theory there may be no stimulation effect. On the other hand, if complete block be established around either pole, the effect may equally fail. I believed that the effect was wanting on the side of the cathode with the weaker currents because the modification of conductivity was slight, and that precisely the reverse was the case on the anodic side with the strong currents. Experiments, of which 52 is a type, were therefore made to determine this point. The nerve was crushed over the galvanometer electrode which was more remote from the polarising circuit. The conductivity here would therefore remain nil. The action current of the unpolarised nerve was, of course, very large, and its amount would depend upon the intensity with which the excitation passed the other galvanometer electrode, which was common to the two circuits. Changes of conductivity around this electrode would be shewn by changes in the amount of the stimulation effect, if the excitability at the stimulated point were constant. With stimulation in the middle of the intrapolar area, the difference between the action of the two poles would appear. It will be seen that the stimulation deflection was always in the same direction in the polarised and unpolarised nerve. It was always diminished just after opening the polarising current, but it was in general far more diminished after the descending than after the ascending current; and with 12 D ↓ passed for 5″ it almost disappeared. With 12 D ↑ passed for 15″ it was also very greatly diminished. This is just the behaviour we should expect, if our theory is correct. And here, as with the intrapolar effect, the amount never exceeds that of the full action stream. The action current is diminished, never reversed. That the cathode after long passage of a strong current leaves behind it a depression comparable to that left behind by the anode, we have already had abundant proofs. Some more will be furnished later on. Experiment 51 shews that the direction of the effect does not depend on the stimulation being median, although the amount may.

VI. OBSERVATIONS AFTER OPENING THE POLARISING CURRENT WHEN THE INTRA- AND EXTRAPOLAR REGIONS ARE LED OFF ALTERNATELY TO THE GALVANOMETER, THE STIMULATING ELECTRODES BEING EXTRAPOLAR.

Fig. 7 shews the arrangements in outline.

FIG. 7.



C is a Pohl's commutator without cross wires by means of which either extra- or intrapolar region could be connected with the galvanometer *G*. *K* is a double paraffin key which closed the galvanometer circuit immediately after opening the battery *B*.
C′ is a compensator to balance the current of rest.

EXPERIMENT 52*a*.

Polarising current.	Time of flow.	Stimulation effect.	
		Extrapolar.	Intrapolar.
5 D ↓	5″	+ 18	− 22
5 D ↓	5″	+ 30	− 22
5 D ↑	1′	− 33	− 26
	in 1′ after opening	− 18	− 39
	2′ more ,,	− 7	− 47
	2′		− 31

Distance I E_1 9 mm.
E_1 E_2 9 ,,
E_2 E_3 0 ,,
E_3 E_4 9 ,,

EXPERIMENT 53.

Polarising current.	Time of flow.	Stimulation effect.	
		Extrapolar.	Intrapolar.
			unpolarised 56
5 D ↓	1′	+ 29	25
	in 1′		36
	2′	+ 13	42
	3′		47
	5′	0	58
			unpolarised 105
10 D ↓	1′		
	in 1′	+ 35	16
	2′	+ 28	25
	3′		34
	4′	+ 25	44
	6′	+ 24	73

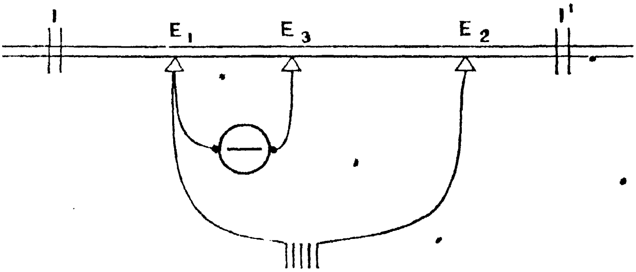
Same distances as in Experiment 52*a*.

Two nerves in reverse order. Cross section on lowest polarising electrode.

I forbear from quoting more of the experiments, as the paper is already unduly lengthened. The general result is that a large extrapolar effect is associated with a small intrapolar effect, and *vice versa*. Thus, in Experiment 52, after 5 D ↑ the extrapolar effect is at its height immediately after the current is opened, and falls continuously away; the intrapolar effect increases for some time after opening the current. This increase would be explained by the fact that the cathodic block does not last so long as the anodic. The former, after such a long closure time as 1′, would at first cut down the excitation greatly; later on the excitation would pass freely into the intrapolar region, but not freely over the anodic area. In Experiment 53 the full action stream in the intrapolar area is never reversed. Immediately after opening the descending current it is greatly reduced, with 10 D almost abolished. It increases continuously with the interval from the opening of the current, and regains its former amount. The extrapolar effect, as before, is at its maximum immediately after opening.

What is the cause of the stimulation effects after opening the polarising current? Are they secondary action currents, such as we assumed in discussing the variations during the flow, or are they due to some change in the capacity of the nerve for polarisation, connected with the functional activity of the axis cylinder? It is not easy to see how the latter hypothesis could explain the results, even if we had any direct evidence in its favour. In the first place, the stimulation effect in the intrapolar area is in the direction of a diminution of the positive polarisation deflection. Assuming the existence of a true positive polarisation, we might suppose that the excitation caused a partial depolarisation. But it would be necessary to suppose further that, after the stimulation had ceased, the positive polarisation was re-established, except so far as it would have fallen away had the nerve not been excited. For separate experiments have shewn that the positive deflection takes as long to disappear when the nerve is stimulated continuously or at intervals, as when it is left alone. Besides, everything seems to shew that this deflection is not due to galvanic polarisation. An increase in the negative polarisation would equally explain the facts, provided that this increase disappeared when the excitation was over. But how are we to imagine an increase in the negative polarisation being brought about, and how are we to reconcile it with what takes place during the flow of the current? Again, admitting that there is an increase in the intrapolar negative polarisation, how shall we explain the decrease in the extrapolar after-currents?

FIG. 8.



If we say that the main anodic current is an action current, as Hermann has done, how shall we explain its negative variation as due to a change in polarisation? If we say that the cathodic after-current is a polarisation current, how shall we account for the fact that stimulation diminishes it, but increases the polarisation current in the intrapolar region? We turn to the explanation first suggested, and ask, (1) Can the stimulation effects be accounted for as secondary action currents? (2) Is there any evidence in favour of the existence of such currents after opening the polarising stream?

As regards the first question, if we assume that after opening the conductivity around both anode and cathode is depressed, but that the depression is greater around the former, the *direction* of the effect will be accounted for in every case. In the intrapolar area there will be a negative stimulation effect; in the extrapolar anodic area, a positive effect; in the cathodic region, a negative effect. Further, in general, the intrapolar effect will be greater when the excitation has to pass the cathode than when it has to pass the anode. The extrapolar effect will be greater, for extrapolar stimulation, on the side of the anode than on the side of the cathode; but this inequality will become less and less marked, the more the current density and time of flow are increased, because the conductivity will in both cases tend to a maximum which, we may expect, will be first reached at the anode. With median intrapolar stimulation, it is not difficult to see that at first the behaviour of the extrapolar effect will be the same; but the time will come when the relations of the two poles will be reversed, for, when the anode has become impassable, the excitation may still be able to pass the cathode. So that the first hypothesis will explain also the *quantitative* relations.

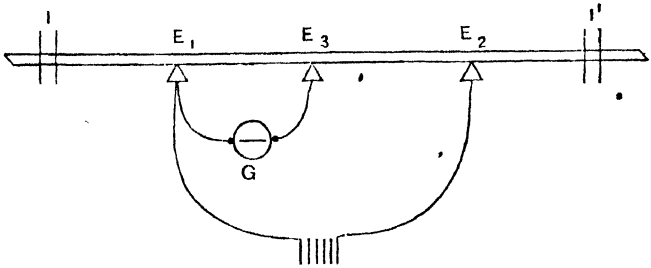
The second question as to the evidence in its favour has been partially answered in answering the first. For it is difficult to see how the facts can be explained without it. Some more direct evidence has been already got from experiments like 52, where median intrapolar stimulation was used, and the negative variation taken as the measure of the intensity with which the excitation was able to pass the poles. Further evidence will be furnished when we come to the muscle contraction experiments. The case stands thus: the phenomena can all be qualitatively explained as secondary action currents, due to differences of conductivity; their quantitative relations can also be so explained; we have proof that such action currents must arise; and finally the effects are never of a higher order than the full action stream from longitudinal surface to cross section. The conclusion does not seem doubtful.

VII. OBSERVATIONS AFTER OPENING THE POLARISING CURRENT, WHEN A PART OF THE INTRAPOLAR AREA IS LED OFF TO THE GALVANOMETER.

This section of the work was an attempt to answer the question: What is the distribution in the intrapolar region of the changes of conductivity left behind by the polarising current? I was very desirous of answering the analogous question for the case during the flow; but I have not been able to see any satisfactory way of doing this, at least with the galvanometer, because the great resistance which would have to be put in the galvanometer circuit, to prevent short circuiting of the current through it, would cut down the stimulation effect too much. After the flow there is, of course, no such difficulty.

In Fig. 9 let E_1 be the anode and let the stimulating electrodes be at I. If the anodic depression extends in full amount to E_3, the intrapolar galvanometer electrode, we ought to get no deflection on stimulating. If the conductivity at E_3 is unchanged, increased, or, in general, greater than that at E_1, there ought to be a negative effect, if the excitation can pass at all. Since we must suppose that in the immediate neighbourhood of the pole, the modifications, whatever they are, which affect the conductivity are most strongly marked, we should in any case expect a negative effect; but, if the resistance of the nerve be eliminated by the introduction of sufficient resistance into the galvanometer circuit, the effect ought to be smaller when a part only of the intrapolar area is led off than when the whole is led off. If E_1 were made the cathode, I being still the place of stimulation, a negative stimulation effect would mean that the conductivity at E_3 was less than at E_1. If this were constantly got, with every position of E_3, the inference would be that any point nearer the anode has a smaller conductivity than any point farther from the anode, that, in other words, the

FIG. 9.



curve of conductivity slopes continuously down from cathode to anode. If stimulation were made at l_1, so that the excitation could not reach the galvanometer area without first passing through a portion of the intrapolar region, the problem would become more complex, because the amount of the stimulation effect would be influenced not only by the state of the piece of nerve in the galvanometer circuit, but also by that of the rest of the intrapolar region. These were the considerations which I wished to test by experiment, both for extrapolar and for intrapolar stimulation.

1. *Stimulating electrodes extrapolar.*

The important point to determine was the influence of the anode. It was arranged that a greater or less portion of the intrapolar region should be connected with the galvanometer, including either of the poles, or neither of them. In Experiment 54 the polarising electrode next the stimulating electrodes, and an intrapolar point were connected with the galvanometer.

EXPERIMENT 54.

Polarising current.	Time of flow.	Polarisation deflection.	Stimulati effect.
6 D ↓	5″	+ 60	0
6 D ↓	very short	+ 75	0
6 D ↑	"	− 110 + 75	− 45
6 D ↓	"	+ 70	− 4
6 D ↑	"	− 90 + 80	− 30
12 D ↑	"	− 90 + 250	− 33
12 D ↓	"	− 58 + 320	− 6
12 D ↑	"	− 95 + 250	− 36
12 D ↓	"	− 50 + 320	0

Length of nerve in polarising circuit 18 mm.
" " galvanometer " 5 "

In Experiments 55 and 56 the polarising electrode most remote from the point of stimulation was so connected. The stimulating electrodes being at the central end, with descending current the anode would be led off in the former case, the cathode in the latter; but there would be this difference, that with the second arrangement the excitation would have to pass a portion of the intrapolar area, before it reached the galvanometer circuit.

EXPERIMENT 55.

Polarising current.	Time of Flow.	Polarisation deflection.	Stimulation effect.
Length of nerve in galvanometer circuit 10 mm.			
5 D ↓	5″	− 151 + 92	− 25
5 D ↑	5″	− 187 + 95	− 31
10 D ↑	5″	− 365 + 208	− 37
10 D ↓	5″	− ∞ + 122	0
5 D ↑	5″	− 194 + 114	− 32
Length of nerve in galvanometer circuit 7 mm.			
5 D ↑	5″	− 215 + 104	− 30
5 D ↓	5″	− 236 + ?	− 27
10 D ↑	10″	− ∞	− 26
	in 30″		− 19
	2′		− 9
10 D ↓	10″	− ∞	0
	in 30″		− 13
	1′		− 16
	2′		− 8
	3′		− 3

Length of nerve in polarising circuit in whole experiment 12 mm.

EXPERIMENT 56.

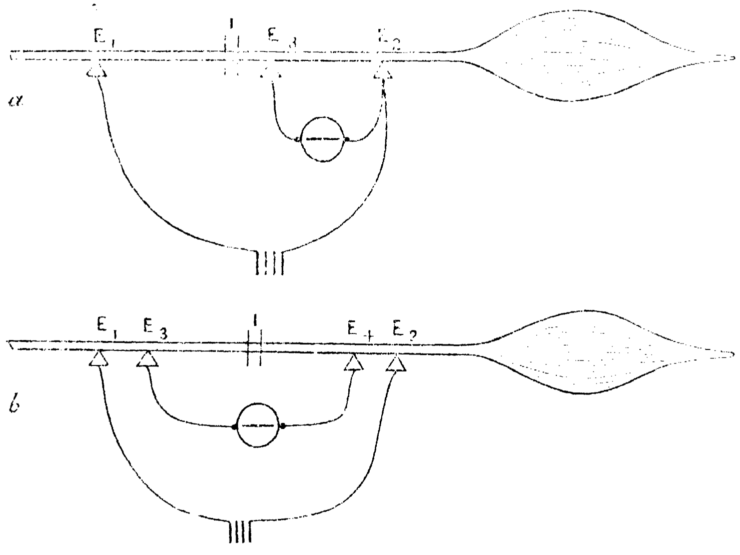
Polarising currency.	Time of Flow.	Stimulation effect.
Galvanometer electrodes coincident with polarising.		
10 D ↑	5″	− 6
10 D ↓	5″	0
Length of nerve in galvanometer circuit 11 mm.		
10 D ↑	5″	− 7
Length of nerve in galvanometer circuit 6 mm.		
10 D ↑	5″	− 4
10 D ↓	5″	0
Length of nerve in galvanometer circuit 3 mm.		
10 D ↑	5″	0
10 D ↓	5″	0
Galvanometer electrodes coincident with polarising.		
10 D ↑	5″	− 6

Length of nerve in polarising circuit in whole experiment 12 mm. A resistance equal to about five times that of the 12 mm. of nerve was introduced into the galvanometer circuit.

In Experiment 54 we see that, when the excitation has to pass the anode, after strong currents, there is no stimulation effect, or a small one in the usual direction. When it has to pass the cathode, the effect is large, and also in the normal direction.

In Experiment 55 the excitation has always to pass through some of the intrapolar region, before it reaches the part led off; but the anode is a more formidable obstacle than even 5 mm. of the nerve next the cathode. Whenever there is an excitation effect at all, it is in the negative direction as usual. Experiment 56 shews how the effect varies in amount with different lengths of nerve in the galvanometer circuit, when the total resistance in that circuit is made so great that the variation of resistance due to the nerve may be neglected. On account of the extra resistance the numbers would have to be multiplied by about 6, in order to be comparable with those of the other experiments. When the anode is next the stimulating electrodes, the effect falls out as before. When the excitation has to pass the cathode the

Fig. 10.



effect diminishes, when any considerable portion of the intrapolar area lies between it and the led off part; and it disappears ultimately.

2. *Stimulating electrodes intrapolar.*

The arrangement is shewn by Fig. 10. Experiment 57 is an example of *a*, Experiment 58 of *b*.

EXPERIMENT 57.

Polarising current.	Time of flow.	Stimulation effect.
5 D ↓	·5″	0
5 D ↑	·5″	− 5
10 D ↓	·5″	− 7
10 D ↑	·5″	− 8
5 D ↑	5″	− 15
5 D ↓	5″	− 1
10 D ↓	5″	− 14
10 D ↑	5″	− 11
12 D ↑	·5″	− 36
12 D ↓	·5″	− 24

EXPERIMENT 58.

Polarising current.	Time of flow.	Polarisation deflection.	Stimulation effect.
5 D ↑	5″	− 81	− 3
18 D ↓	very short	− 82 + 121	− 11
18 D ↑	”	− 119 + 126	− 10
10 D ↓	10″	− ∞	− 3
10 D ↑	10″	− ∞	− 3
18 D ↑	very short	− 88 + 130	− 12

Nerve crushed over polarising electrodes.

18 D ↓	very short	− 122 + 165	− 8
18 D ↑	”	− 148 + 160	− 15
18 D ↓	”	− 129 + 165	− 9
18 D ↑	”	− 132 + 160	− 13

Dist. between polarising electrodes 36 mm.

Distance between each galvanometer electrode and the corresponding polarising electrode 5 mm.

Stimulating electrodes in middle of intrapolar region.

Two nerves in reverse order.

Experiment 59.

Polarising current.	Time of flow.	Polarisation deflection.	Stimulation effect.
18 D ↓	very short	−145 +202	−11
18 D ↑	”	−161 +145	0

The lower polarising electrode was also a galvanometer electrode, and the nerves were crushed over it.

Distance of upper galvanometer electrode from upper polarising 5 mm.

Distance between polarising electrodes 32 mm.

Two nerves in reverse order.

18 D ↑	very short	−110 +155	0
18 D ↓	”	−137 +170	−13

It was desirable to find whether the failure of the effect with extrapolar stimulation, when the anode was next the stimulating electrodes, was due to a block at the anode. If so, the effect would be expected to shew itself with intrapolar stimulation. Experiment 57 shews that even with the strongest currents used, the stimulation effect does not disappear after descending current. It rather increases with the strength. In general when the anode is in the led off area, the effect is greatest.

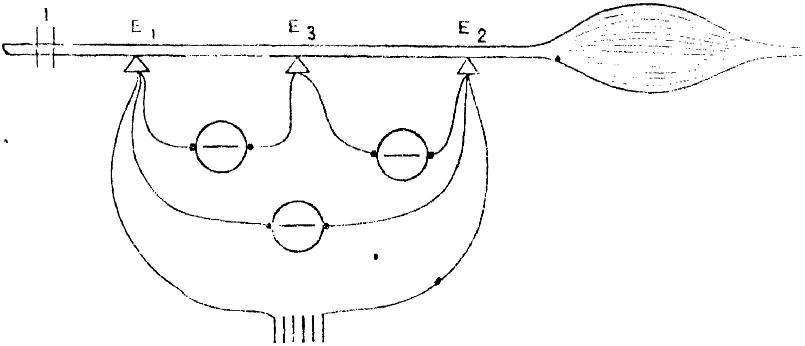
In Experiment 58, as might be expected, there is practically no difference between the two directions, at any rate before the nerves were crushed. The difference after crushing is probably due to the crushing not having been uniform over the two electrodes. In Experiment 59, when the anode corresponds to the dead piece of nerve, the stimulation effect is absent. I have never, however, under these circumstances, been able entirely to get rid of a positive polarisation deflection, when the current was strong and closed only for a short time.

Before discussing further the results of this section, it will be well to complete the account of the differential investigation of the intrapolar region, by giving some examples of experiments where different parts of the region were compared with each other.

VIII. OBSERVATIONS AFTER OPENING THE POLARISING CURRENT, WHEN THE WHOLE INTRAPOLAR REGION AND PART OF IT OR TWO DIFFERENT PARTS OF IT ARE LED OFF SUCCESSIVELY TO THE GALVANOMETER, THE STIMULATING ELECTRODES BEING EXTRAPOLAR.

Fig. 11 is a scheme of the arrangement. Of course one galvanometer only was required, the different regions being thrown on to it successively. The cathode was always next the point of stimulation, so as to avoid the anodic block. The electrode E_3 was exactly in the middle of the intrapolar area.

Fig. 11.



In Experiments 60 and 61 two nerves in reverse order were used; in Experiment 62 a single nerve, but only the part below the thigh branches.

EXPERIMENT 60.

Polarising current.	Time of Flow.	Polarisation deflection.			Stimulation effect.		
		Anodic half.	Cathodic half.	Whole intrapolar region.	Anodic half.	Cathodic half.	Whole intrapolar region.
12 D	very short	− 238 + ∞	− 216 + 227	− ∞ + ∞	− 12	− 12	− 17
5 D	”	− 85 + 161	− 111 + 31	− 91 + 134	− 12	0	− 7
5 D	”	− 98 + 183	− 129 + 36	− 152 + 154	− 9	0	− 9
3 D	5″	− 242 + 102	− 268	− 344 + 45	− 7	0	− 5
12 D	very short	− 190 + ∞	− 212 + 195	− ∞ + ∞			− 14

EXPERIMENT 61.

1 D	15″	− 39	− 35	− 51	− 3	0	− 4
3 D	5″	− 95 + 27	− 148	− 155	− 5	− 1	− 3

EXPERIMENT 62.

5 D	·5″	− 49 + 131	− 37 + 32		− 11	− 6	
10 D	·5″	− 66 + 298	− 92 + 131		− 16	− 13	
5 D	5″	− 169 + 193	− 262 + 46		− 21	− 7	
5 D	15″	− 38 + 79	− 350		− 28	− 14	
10 D	3½″	− ∞ + 172	− ∞ + 104		− 39	− 21 − 30	
10 D	15″	− ∞ + 143	− ∞		− 33	− 24	

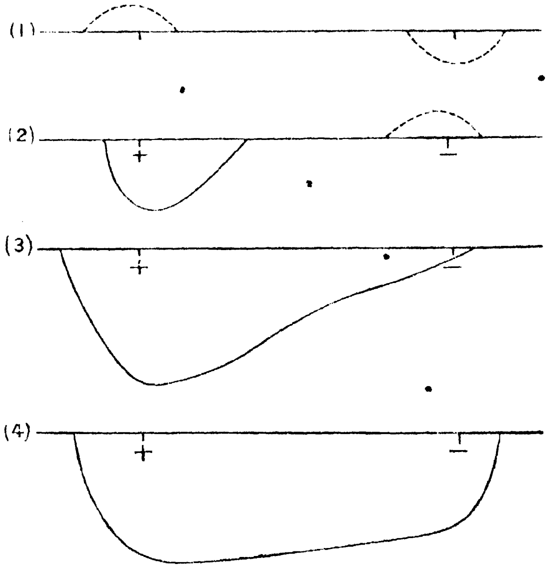
From the results of Section VIII. it will be seen that except with the strongest currents the effect is greater when the galvanometer is connected with the anode than when it is connected with the cathode. In the latter case, with weak currents it is often absent. When this happens, we must suppose that the modification of conductivity is entirely confined to the anodic half. Experiment 56 makes it probable that in these circumstances the block is really limited to the immediate neighbourhood of the anode. For even with 10 D closed for 5″ an effect was still got when *half* the nerve was led off, although the excitation was quite unable to pass the anode, and it was necessary to reduce the led off region to one-fourth of the whole intrapolar area to get rid of the effect altogether. In this part of the polarised area we might conclude that the conductivity was abolished. The preponderance of the anodic half shews itself in spite of the fact that the excitation has to traverse the whole cathodic area first. The greater the stimulation effect in the latter, the deeper must the depression of conductivity, on our theory, have stretched into the cathodic region—the more difficult must it, therefore, be for the excitation to reach the anodic half at all. Nevertheless, the anode nearly always bears off the victory, another sign that it is near the anode itself that the modification is most marked. The time comes, however, as current density and time of flow are increased, when the excitation can no longer break through into the anodic region, or can do so only in small intensity; and then the relations of anode and cathode are reversed. The positive polarisation deflection is also greatest in the anodic region as Hermann has observed, and is often only seen there. It is not only greater in the anodic area than in the cathodic, but also greater than when the whole intrapolar region is led off. It is difficult to resist the conclusion that there is some connection between this distribution of the positive polarisation and that of the stimulation effect. If we explain the former as due to an excitatory process localised around the anode—most marked here, it may be, and fading away in the intrapolar region—it seems natural to suppose that whatever checks the spread of this excitation, be it an independent effect of the current, perhaps electrolytic, or be it a secondary action of the excitation itself, will also oppose the propagation of an impulse set up at any part of the nerve. The barrier which keeps the anodic excitation from breaking out will keep the external excitation from breaking in. But the hypothesis of lowered conductivity around the anode, which we have used all along, is just a different way of stating the same thing. Whatever the nature of the inhibition may be, it seems certain that its cause does not lie in the cathodic region. This would, indeed, explain the existence, although not the distribution of the positive polarisation deflection, but it is contradicted by the results of stimulation; and I think we may safely conclude that the excitation which gives rise to that deflection is confined to the anodic region by something in that region itself.

In Experiments 60 and 61 it should be remembered that the difference of resistance in the different observations must be allowed for. On our hypothesis, when this is done, the sum of the stimulation effects in the two halves should be about equal to the effect in the whole intrapolar region. The ratio of the resistances would be about 3 : 5, the galvanometer being over 7000 ohms. The sum of the two partial effects must therefore be multiplied by $\frac{3}{8}$, before being compared with the total effect. The discrepancy is not very great. Neither, however, is the agreement very striking. In observations of this kind, where three successive experiments on an object like a nerve have to be compared, a striking numerical agreement would be more suspicious than convincing. I do not lay any stress on the point. If the effect depended on something uniformly distributed along the nerve, the same behaviour would be expected. But seeing that the cause of the effect is obviously not so distributed, it may be of some little interest.

I conceive, from a consideration of all the experiments, that the curves representing the conductivity after opening the polarising current run somewhat as in Fig. 12, (2) being the effect of a moderate current, (3) of a strong, and (4) of a very strong current.

During the flow the same curves will represent the probable state of affairs, if the position of the anode and cathode be interchanged; but a much smaller current density will give a measurable effect during the flow, and therefore corresponding stages will not be associated with equal current densities. (1) represents what probably happens with the weakest currents, namely a rise of conductivity around the cathode, a diminution around the anode, while the polarising current is flowing. (1) is only partly based on my own work. Whether it would be a correct representation of the after-effects if anode and cathode were interchanged as with the stronger currents, I am unable to say.

FIG. 12.



IX. EXPERIMENTS ON MUSCULAR CONTRACTION WITH INTRAPOLAR STIMULATION.

1. *During the flow of the polarising current.*

In 1873[1] Hermann made experiments on muscle contraction with electrical stimulation in the intrapolar region, which included almost the whole nerve from the vertebral column to the muscle. He used only a single nerve. He found that with the descending current, the stimulating electrodes being in the upper part of the lower half of the intrapolar area, the contraction was always less than before polarisation, or might disappear altogether; with the ascending current it was always greater. He saw in this a proof of the law of polarisation increment. The fallacy which might arise from the variation of the cross section of the nerve, and the consequent variation of current density from point to point, was afterwards remarked by Hermann himself.[1] He found that, if the thin lower part of the nerve had another piece of nerve laid on it so as nearly to equalise the cross section, the above-mentioned behaviour was reversed. When he used only the piece of nerve below the thigh branches, he could find no point in the lower half the stimulation of which gave his former results. Without knowing of Hermann's work at the time, I made experiments in connection with the galvanometric observations detailed above, but on different lines. All that I wished to determine was the *relative* effect of the two poles on the conductivity. To determine the *absolute* effect would have been very desirable, but to do this it would be necessary either to shew that changes of excitability might be neglected, or to measure their numerical amount. This I despaired of doing, and therefore confined myself to the more definite task. Some of the experiments were made with a single nerve, the intrapolar area varying in cross section. But these I decided to control and supplement by observations with approximately uniform cross section, obtaining this in some cases by using only the pieces of the sciatic below the thigh branches, but more frequently by laying two nerves of the same frog together in reverse order, both muscles being left attached. In the latter case the cross section would not be perfectly uniform, but would be nearly so. When the two preparations were found to have a nearly equal excitability, which unfortunately did not generally happen, the contractions of the two muscles caused by the same stimulus were simultaneously registered. This was probably the most accurate method of all, because there could be no question of differences of excitability. In order to eliminate these when two successive observations had to be taken with the current flowing in different directions, the stimulating electrodes were placed exactly in the middle of the intrapolar area. Four narrow transverse grooves were made in a small slab of paraffin, two for the stimulating electrodes and two for the polarising. The grooves were filled with clay, and between them the paraffin was hollowed out, so that the whole formed a platform on which the nerves lay without touching it except at the grooves. The two central grooves were very close together, and each was separated by the same interval from the corresponding groove of the outer pair. The non-polarisable electrodes were connected with the grooves. A rather more convenient arrangement, which allowed the distance between the polarising electrodes and the position of the stimulating pair to be easily varied, was got by fixing a paper scale on a paraffin slab, to the edge of which the clay points of the electrodes carrying the nerves were adjusted. I introduced much more resistance into the secondary circuit than Hermann seems to have thought necessary, namely the large resistance *B*.

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[1] Pflüger's *Archiv*, Bd. VII., p. 323.

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[1] *Ibid.* p. 497.

For the weaker currents the strength of stimulus was sought which was just less than that necessary to give a minimal contraction, when the cathode was next the muscle. Then a sufficient interval was allowed (in quoting the experiments this is not noted to save space), and with the current in the opposite direction, it was observed whether contraction occurred on stimulating. For the stronger currents the stimulus was adjusted so as to give contraction when the anode was next the muscle, and the current was then reversed after an interval, and stimulation again made. The reason of the difference will appear when the experiments are looked at. It will be seen that *up to a certain strength of current a stimulus will give contraction when the cathode lies next the muscle, which will give no contraction when the anode is in that position. Above this strength the reverse holds, and a stimulus which is followed by contraction when the excitation has to pass the anode, evokes no response when it has to pass the cathode.* The change is not very gradual, but can hardly be called abrupt. When the cathodic block first appears, it can be overcome by increasing the strength of the stimulus. It does not, however, require a great addition to the current density to make the cathode impassable for any stimulus within my limits, when once the block has become pronounced. Moreover, a block which can be broken down at first may become total, if the current be left a little longer closed. With the very weakest currents I have found no difference between the poles; but the excitability of the stimulated point seems to be increased; for a stimulus which caused no contraction in the unpolarised state causes one when the current is closed, the direction of the polarising current being indifferent. For convenience we may call this the first stage, the others the second and third respectively. Hermann seems only to have met with the third stage when he used a single nerve, and stimulated in the lower half. I found the second very constant with median stimulation even with a single nerve, although not so well marked as with the double nerve; but the third stage is undoubtedly reached much earlier in this case than with uniform cross section, even where there is no extra resistance in the nerve circuit, and where, therefore, the same E. M. F. gives practically the same average density of current when the distance between the polarising electrodes is the same. But with a single nerve we must remember that the density of the current in the thin part below the thigh branches will be several times as great as at the plexus. If we could be sure that the excitability was the same at the stimulated point for both directions of the current, this would introduce no fallacy in comparing the effect of the two poles. But in comparing experiments made on the single nerve with those made on the double nerve, it must be taken into account. Where I used the single nerve, far less of the thin part was included in the polarising circuit than of the plexus. Accordingly, if we call the cross section at the upper electrode a, we may assume that a given E. M. F. E would give a current a little less than KaE, where K is a constant depending on length and specific resistance of nerve. Let b be the cross section at the lower electrode; then the density here would be a little less than $\frac{Ka}{b}E$. Now take the case of the double nerve. Here the cross section at the electrodes would be $a+b$, and at no part would it differ much from this. An E. M. F. E would give a current $K(a+b)E$, and the density at either electrode may be called KE. The density at the lower electrode in the case of the single nerve would therefore be nearly $\frac{a}{b}$ times that with the double nerve. I suppose it would not be above the mark to put $\frac{a}{b}=3$ in most cases. The third stage might, therefore, be expected to begin in the single nerve with about $\frac{1}{3}$ of the E. M. F. for which it first shews itself with the double nerve; any change of excitability at the point of stimulation being left out of account.

I proceed to quote a few of the experiments. Eperiment 66 is an example of chemical stimulation at the middle point. A drop of strong brine was applied and left a little time, and then, before tetanus had appeared, a current of 1 D ↑ was passed. Tetanus appeared at once, and disappeared on opening the current. With the descending current there was no tetanus. With 2 D the stimulus was apparently too weak to give contraction with either direction. In Experiment 67 mechanical stimulation was used, also at the middle point.

Experiment 67 *b* is an instance with marked inequality of cross section. Below 1 D Rh 900 the descending current seems more favourable than the ascending. But with 1 D and above it the opposite is the case. Experiment 67 *a* is an example where the two muscles were observed simultaneously. As in all cases where two nerves were used, the polarising electrodes were at the same distance from the point of entrance of the nerves into their muscles, and therefore the cross section and current density at them would be equal. In some of the experiments it was noted whether contraction occurred at make or break of the polarising current or at both. The second stage as a rule passed into the third with a current which was either Pflüger's "strong" current or a strong "medium."

EXPERIMENT 63.

Polarising current.	Muscle contraction on stimulation.	Stimulus.	Muscle contraction on making or breaking polarising current.
1 D Rh 1000 ↓	No	100	
" ↑	C	"	
2 D ↓	No	90	C at M and B.
2 D ↑	C	"	C at M.
3 D ↓	No	80	
3 D ↑	C	"	
4 D ↓	No	"	C at M.
4 D ↑	C	"	C at M and B.
5 D ↑	No	"	C at M.
5 D ↑	C	"	C at B.
5 D ↓	No	0	
4 D ↓	No	0	
3 D ↓	No	0	
2 D ↓	No	0	
1 D ↓	large C	0	
7 D ↑	C	20	C at B.
7 D ↑	No	40	
7 D ↑	C	30	

Distance between polarising electrodes 28 mm. Two nerves in reverse order. C means contraction. No means none. M and B mean make and break of polarising current.

EXPERIMENT 64.

1 D Rh 1000 ↓	No	80	C at M.
" ↑	C	"	"
1 D Rh 500 ↓	C	103	"
" ↑	No	"	"
1 D Rh 1000 ↓	C	"	"
" ↑	No	"	"
1 D whole Rh ↓	C	"	"
" ↑	No	"	"
2 D whole Rh ↓	C	"	"
" ↑	No	"	"
3 D ↓	C	"	"
3 D ↑	No	"	"
4 D ↓	C	65	C at M and B.
4 D ↑	No	"	"
5 D ↓	No	48	"
5 D ↑	C	"	"
6 D ↓	No	47	"
6 D ↑	C	"	"

Two nerves in reverse order. Resistance A in nerve circuit. Distance between polarising electrodes 31 mm.

EXPERIMENT 65. Two nerves in reverse order.

Polarising current.	Muscle contraction on stimulation.	Stimulus.	Muscle contraction on making or breaking pol. current.
1 D Rh 1000 ↓	C	74	
” ↑	No	”	
1 D whole Rh ↓	C	70	C at M and B.
” ↑	No	”	C at M.
1 D ↓	C		
1 D ↑	No		
2 D (no resistance) ↓	No	66	
2 D ↑	C	”	
4 D ↓	No	50	C at M; small C at B.
4 D ↑	C	”	Small C at M; C at B.

Distance between polarising electrodes, 31 mm. Resistance A in nerve circuit.

EXPERIMENT 66.

Polarising current.	Chemical stimulation.
unpolarised	No tetanus
1 D ↑	Tetanus
1 D ↓	No
1 D Rh 2000 ↑	Tetanus
” ↓	No
1 D Rh 1000 ↑	Tetanus
” ↓	Tetanus not so strong as with ↑
1 D Rh 500 ↑	Tetanus
” ↓	Tetanus not so strong as with ↑
1 D Rh 300 ↑	No
” ↓	Tetanus (strong)
	The tetanus is now continuous in unpolarised preparation.
1 D ↑	Tetanus increased
1 D ↓	No
2 D ↑	No
2 D ↓	No

Only a single nerve used.

EXPERIMENT 67.

Polarising current.	Mechanical stimulation (single stimuli).
1 D ↑	C
1 D ↓	C
2 D ↑	C
2 D ↓	No
3 D ↑	C
3 D ↓	No
4 D ↑	C
4 D ↓	No
6 D ↑	C
6 D ↓	No
1 D Rh 1500 ↓	C
” ↓	No

EXPERIMENT 67*a*. Two nerves in reverse order.

Polarising current.	Muscle A.	Muscle B.	Stimulus.
unpolarised	C	C	45
5 D	↑ C	↓ No	,,
	↓ No	↑ C	,,
5 D	↓ No	↑ C	30
	↑ C	↓ No	,,
5 D	↓ No	↑ C	0
	↑ C	↓ No	,,
unpolarised	C	C	50

Here the excitability of the two preparations was nearly the same, and it was therefore possible to take observations on both muscles at the same time. The arrows give the direction of the current in the nerve of the muscle whose contraction is referred to.

Distance between pol. electrodes 34 mm.

Resistance A in polarising circuit.

EXPERIMENT 67*b*.

Polarising current.	Mechanical stimulation (single stimuli).
1 D Rh 200 ↑	No
,, ↓	C
1 D Rh 900 ↓	C
,, ↑	No
1 D ↓	No
1 D ↑	C
2 D ↓	No
2 D ↑	C
3 D ↓	No
3 D ↑	C
4 D ↓	No
4 D ↑	C
5 D ↓	No
5 D ↑	C
6 D ↓	No
6 D ↑	C
7 D ↓	No
7 D ↑	C
8 D ↑	C
1 D Rh 200 ↓	C
,, ↑	No

Only a single nerve. Upper polarising electrode on plexus, lower just below thigh branches.

In interpreting these results it is necessary to be careful. If we could be sure that the excitability at the point of stimulation was always the same for the two directions, or in other words that the excitation at its start had the same intensity, then it would, I think, be impossible to doubt that the impulse does alter in amount in passing along the polarised nerve. In any one experiment it is of course impossible to be sure of this, unless, as Hermann has suggested, changes of excitability in Pflüger's sense are apparent only and not real. But in a series it is highly improbable that the error should be always, or even nearly always in the same direction. So far, indeed, as the third stage is concerned, the difference between the two directions soon becomes so marked that accidental errors due to differences of excitability may, I think, be neglected. The stimulating electrodes may be placed far from the middle of the intrapolar region, and either above or below it, without altering the results in a qualitative way at least. On the whole I do not see how for this stage, without considering the galvanometric evidence at all, we can avoid the conclusion that the conductivity around the cathode is less than that around the anode.

I have already said that, after the cathodic conductivity has once begun to decline, the curve falls sharply till the pole is impassable for the strongest stimulation. For the anode the fall is much more gradual, and a moderate strengthening of the stimulus suffices to bring out the contraction which has just disappeared. If we increase the current now a little the contraction will again fall out, to be again restored on strengthening the stimulus and so on. Finally the time will come, as we saw that it came in the galvanometric experiments, when even with the ascending current no excitation will pass with the strongest stimulation. But this condition will gradually blend into what preceded it. This seems to me to support the view, for which we have already seen other evidence, that only around the cathode is there a truly polar block during the flow. The gradual failure of effect with ascending current may be explained as due to a continuous diminution of excitability at the point of stimulation, or to a gradual spread of the depression of conductivity from cathode to anode along the intrapolar area, or to both. So far I think we are on safe ground. Neither is there any difficulty with the first stage. We should hardly expect to find well developed differences of conductivity with the weakest currents used. At any rate there must be a limit below which the method would not be delicate enough to detect them.

But it is more difficult to deal with the phenomena of the second stage. Here the contraction seems to be favoured by the descending current. If the explanation is to run on the same lines as before, this would imply that, for these currents, the conductivity is greater in the cathodic region than in the anodic. And there is a fact which supports this view. It was found by Rutherford and by Wundt that the velocity of the nerve impulse is increased in the cathelectrotonic and diminished in the anelectrotonic area, when the polarising current is weak and not closed for too long a time. It cannot, of course, be assumed that the velocity of the impulse, and its intensity must necessarily be affected in the same sense. In the unpolarised nerve we do not know that the intensity of the impulse affects its velocity at all. But some conditions which diminish the latter diminish the former also, cooling the nerve for example. And Engelmann has found[1] that in the ureter the velocity and the amplitude of the contraction wave rise and fall together, at least within certain limits, the alterations produced by a voltaic current being confined to the intrapolar area, beyond which also until a comparatively recent period galvanic electrotonic phenomena have not been observed in muscle. I am far from saying that this can be extended to the nerve impulse without further parley. But it is a hint that in nerve also an excitation which passes more quickly through a part will pass with less loss, or greater gain. v. Bezold, working with stronger currents than Rutherford and Wundt, found that the velocity of the nerve impulse is diminished both in the anodic and cathodic area. This corresponds with our third stage, if for velocity we read intensity. It would be interesting to know if the velocity, like the intensity, is more diminished around the cathode than around the anode.

The galvanometric observations in the intrapolar region give no indication of the state of matters which we have supposed to exist during the second stage. The stimulation effect is always positive even with the weakest currents. A secondary action current caused by a relatively increased cathodic conductivity would be negative. Still it is unlikely that the whole intrapolar effect during the flow is due to an action current under any circumstances, and the part of the positive effect which for want of a better name we may call the electrotonic part, might be strong enough to mask a weak secondary action current. The stage where the galvanometric effect is about equal for the two directions would then pass directly into that where the decline of cathodic conductivity diminishes the effect for stimulation on the side of the cathode. As was stated on p. 32, a positive extrapolar variation was sometimes got in the cathodic area with weak currents and especially with fresh nerves. The conditions under which it appeared are the same as those under which the velocity of the impulse is increased. If we remember that the true electrotonic variation is in the opposite direction, it will not appear surprising that this positive effect should be always small, and should only occasionally appear at all. Another point which must be considered is this, that the contraction of a muscle is a far more delicate reagent for the detection of a nerve excitation than a galvanometer deflection. Finally it ought not to be lost sight of that the nerve endings in the muscle, or even the muscle itself, may be affected by the polarising current, and this may have an influence on contraction, as well as the conductivity or excitability of the nerve trunk. There would of course be nothing corresponding to this in the galvanometric observations.

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[1] Pflüger's *Archiv*, Bd. III., p. 247, &c.

It may be said that there is not enough evidence for us to conclude that during the second stage the conductivity around the cathode is increased. What evidence I have found has been stated. It is of some weight but not perfectly conclusive. The evidence shewing the relative depression of conductivity around the cathode during the first stage is very much stronger, so strong that it does not seem easy to doubt it. There is one objection, however, which ought not to be passed over. It may be said it is with comparatively strong currents that most of this work has been done; are the effects observed truly physiological, or are they due to injury of the tissue? It is a legitimate question. If the effects were due to permanent injury, the observation of them would not be without value, provided they were constant effects. In this investigation, however, I did not wish to study the behaviour of currents of injurious strength. The nerve was therefore tested as to its condition at intervals throughout every experiment. The assumption was, that a nerve which at the end of an experiment shewed, something like the same behaviour qualitatively and quantitatively as at the beginning, had not been injured so far as the properties on which the particular effect depended were concerned. In a certain sense it is no doubt true that a nerve whose conductivity is completely abolished is injured; but the change is within physiological limits, if, in an isolated preparation, the original conductivity is afterwards restored.

2. *After opening the polarising current.*

For the after-effects of the current the arrangements were the same as for the effects during the flow, except that there was a break in the circuit of the secondary coil which was not closed till the polarising circuit had been opened. Further, it was not necessary to have the resistance *A* in the latter circuit, but the large resistance *B* was still kept in the former, to prevent the polarisation currents from passing. After opening the polarising stream, stimulation was at once made, and it was then repeated, sometimes at intervals of 2″, sometimes at intervals of 5″, or even at a greater interval where the block lasted a long time. Experiments 68 to 71 are examples.

EXPERIMENT 68.

Polarising current.	Time of flow.	Effect on muscle.	Stimulus
		max. C	40
6 D ↓	5″	max. C	
6 D ↑	5″	min. C	
12 D ↓	·5″	max. C	
12 D ↑	·5″	No for 8″	
		max. C. in 25″	
		max. C	38
6 D ↓	10″	max. C	
6 D ↑	10″	No for 5″	
		max. C in 15″	
		max. C	37
12 D ↓	5″	min. C at once	
		max. C in 2″	
12 D ↑	5″	No for 9″	
		max. C in 30″	
		max. C	38
12 D ↑	very short	No for 15″	
12 D ↓	,,	max. C	
		C	40
6 D ↑	very short	No for 10″	
6 D ↓	,,	C	

Two nerves reverse order. Resistance *B* in secondary circuit. Intrapolar area 31 mm. Max. means maximal. Min. means Minimal.

EXPERIMENT 69.

		min. C	42
4 D ↑	10″	No for 3″	,,
		min. C	,,
4 D ↓	10″	large C	,,
		min. C	43
4 D ↓	10″	large C	,,
4 D ↑	10″	No for 18″	,,
		max. C	35
12 D ↓	15″	No for 10″	
		max. C in 5″ more	
12 D ↑	15″	No for 70″	
		max. C in 60″ more	
		max. C	33
12 D ↑	very short	No. for 9″	
12 D ↓	,,	max. C	,,

EXPERIMENT 70.

Polarising current.	Time of flow.	Effect on muscle.
		max. C in unpolarised prep.
8 D ↓	5″	C
8 D ↑	5″	No for 55″
12 D ↓	1″	C
12 D ↑	1″	No for 30″
		max. in 30″ more

Only one nerve.

EXPERIMENT 70*a*.

Polarising current.	Muscle A.	Muscle B.	Stimulus.
unpolarised	C	C	55
4 D 10″	↓ C	↑ No for 2″	Resistance *A* in polarising circuit.
6 D 10″	↓ C	↑ No for 4″	
6 D 1′	↓ C	↑ No for 4″	
10 D ·5″	↓ C	↑ No for 11″	
10 D 5″	↓ C	↑ No for 10″	
10 D 5″	↓ No for 5″	No for 60″	

Distance between polarising electrodes 34 mm.

The arrows give the direction of the current in the nerve belonging to the corresponding muscle.

EXPERIMENT 71.

Polarising current.	Time of flow.	Effect on muscle.
1 D ↓	20″	C
1 D ↑	20″	No for 5″
1 D ↓	20″	C
1 D ↑	20″	No for 10″

Single break shocks. Only one nerve.

The results of Section IX., 2 may be summarised as follows:

1. With a very short time of closure, *a.* If we adjust the stimulus so that contraction shall occur immediately on opening the descending current, there will be no contraction for some time after opening the ascending current. *b.* If we adjust the stimulus so that contraction shall not occur for some time after opening the descending current, it will take a longer time to appear after the ascending current.

2. With a longer time of closure, *a* always holds; but, if the stimulus be made so weak that the interval of inefficiency after the descending current is more than a few seconds, the difference between the two directions is not so marked. A strong stimulus is more suitable for bringing out the difference than a weak.

3. If we determine the strength of stimulus which is just necessary to give contraction immediately after opening, it will come out greater for the ascending than for the descending current.

4. When an interval has existed during which the stimulus has been inoperative, the contraction does not reach its original height for some time after the stimulus has again become effective; and this time is longer after the ascending than after the descending current.

The stronger the stimulus the shorter, as might be expected, is the interval between opening the current and the appearance of contraction.

Not infrequently a preliminary stage was noticed with the weaker currents, where a stimulus could be found which gave contraction at once after the ascending current, but not for some time after the descending. This would correspond to the second stage during the flow, but it was very far from being as constant; and, without further investigation, it is not possible to say if such a stage really exists; or, if it does exist, how it is to be explained. With the stronger currents the above results were very constant both for the double nerve and the single nerve preparation; and also, as Experiment 70*a* shews, when two muscles were observed at once.

I do not see how the conclusion can be avoided that after opening the polarising current a rapid or perhaps instantaneous reversal of the relations of the poles takes place.[1] With a short time of flow the cathodic block is at once resolved on opening, and the anodic block is at once established. With a longer time of flow the conductivity is still depressed in the first moments after opening throughout the whole intrapolar region, but the maximum of the depression is found at the anode, while before the opening it was at the cathode. But what else has happened? The anode has itself become the seat of an opening excitation, probably lasting. The cathode was on closure the seat of excitation, a closing tetanus, perhaps, for the cathodic region of the nerve, if not for the muscle. Where the polar excitation reigns, there reigns also the polar inhibition which blocks the external impulse. Is there any connection between the inhibition and the excitation? It will not do to say that the nerve is exhausted by the latter, if it persists. The problem is, why does this external excitation not break its way along the nerve? It is notorious that it does not do so except in special cases. A positive polarisation deflection is constant, a Ritter's tetanus is occasional. Is it not obvious that there is something which hems the internal excitation in? And this something, does it not also prevent the passage of the external excitation? We call the modification which does this a defect of conductivity. It is a convenient name, it is not an explanation. The relation between the conductivity of a nerve at any point and the excitability has been often discussed. It has been said that there is no necessary connection between them. It has been said that they are the same. I confess I do not see the cogency of the reasoning of that school which says that the conductivity and excitability *must* be functions of the same variable, because we cannot conceive of the nerve impulse being propagated except as an excitation of successive nerve sections by each other. Why not? Is the origin of any excitation a process like the propagation of it? I will not speak of artificial stimulation, as we see it in physiological laboratories. If I did, I might ask what analogy there is between the shattering of a nerve trunk by mechanical irritation, the electrolysis[1] of it by electrical irritation, and the propagation of the impulse from section to section of the axis cylinder.

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[1] This might be explained, as Grützner has explained the break contraction, by considering the polarisation current as a new current in the opposite direction to the polarising stream, and closed as soon as the latter is opened. It would lead us, however, too far from our subject to discuss this just now.

But I will take the case of physiological excitation of an axis cylinder process by a nerve cell, or of an end organ such as the retina by light. The two cases are of course different, but let us take the most unfavourable one. Does anybody assert that the active chemical processes in the nerve cell extend themselves to the axis cylinder process or the nerve trunk, when the impulse passes? But it will be said there is no breach of continuity; whatever processes take place in the active motor cell slide insensibly into those which take place in the active motor nerve. But where is the meeting place of the activity of the cell and the activity of the nerve, for that is the point of stimulation? Somewhere or other something which is not the nerve impulse starts the nerve impulse in the nerve. That is the point. Somewhere or other between the layer of rods and cones and the first beginnings of the axis cylinder fibrils of the optic nerve the real retinal stimulus does its work. But never again between retina and brain, between motor nerve cell and muscle, can the stimulus which a nerve section gets from its neighbour be the same as that which started the impulse. It is always now a nerve impulse which precedes a nerve impulse. I do not wish to labour this point. I do not say now that excitability and conductivity are separable properties. What I say is that we know nothing in the manner of propagation of a nerve impulse which goes against such a supposition. We know much which supports it. A good résumé of this evidence is given in a paper by E. Hirschberg[2] published in 1886. The most recent work on the subject has come from the Physiological Institute in Berlin.[3] Carbonic acid gas when apppplied locally to the nerve acts as if it depressed the excitability while leaving the conductivity unchanged. This was first shewn by Grünhagen. Alcohol has now been found to have the opposite effect. Gad, under whose supervision the work was done, indeed rejects the notion of separating conductivity and excitability, chiefly for the reason against which I have been arguing. He thinks the results may be explained by supposing that the nerve has what he calls a transverse irritability which is diminished by carbonic acid but not by alcohol. I do not propose to discuss the merits of this hypothesis, nor the interesting experiments on which it is grounded. These experiments leave the general question where it was. But it seems to be as well established as anything in electro-physiology, that during the flow, the conductivity around the cathode is diminished, at least if the current is of more than very moderate strength, and that this diminution may go on to entire impassability. This was first shewn by Hermann. It has been repeatedly confirmed. Abundant evidence of its truth has presented itself in this research. It is stated on good authority that around the cathode the excitability is increased even with strong currents. Tigerstedt found that this was true on the very pole itself with mechanical stimulation. And it is very likely that an excitation starting at the cathode will pass more easily through the remaining part of the region of depressed conductivity, than if it started with the same intensity somewhere in the intrapolar area. For Werigo found that it did not need a very strong current to block the impulse altogether for intrapolar stimulation at a little distance from the cathode. The same thing has been noticed by me in experiments where the stimulating electrodes, instead of being median, were placed at different parts of the intrapolar area. Even for the extrapolar cathodic region with descending current Engelmann[1] found that the excitability, as measured by the height of the contraction, only increased to a certain limit, as the polarising current was increased in strength, and then declined, passing on into a depression of excitability, which was more marked the nearer to the pole the point of stimulation was. When this was removed farther and farther from the pole, the depression gave place to increase of excitability. This observation, which is very difficult to explain on the ordinary view, becomes at once intelligible, if we suppose that, in addition to the cathelectrotonic increase of excitability in the whole cathodic region, there is a cathodic depression of conductivity which spreads for some distance outside the pole.

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[1] Is electrolysis the essence of electrical stimulation? This is by no means certain. There are considerations which suggest that it may be in certain cases at least a static effect.

[2] Pflüger's *Archiv*, Bd. XXXIX., p. 75.

[3] du Bois Reymond's *Archiv*, p. 350, 1889.

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[1] Pflüger's *Archiv*, Bd. III., p. 403.

It may be said, it is not unlikely that the excitability is really diminished with strong currents near the pole. I have nothing to object. But it is certain that decrease of conductivity does not advance *pari passu* with decrease of excitability, for, if it did, the former would shew itself soonest and most decidedly in the anodic region.

To sum the matter up, there seems to be no doubt that the conductivity is depressed around the cathode during the flow except with weak currents. There is very strong evidence that the excitability is increased. What are we to say? That both things are true, and may be true at the same time. It is not difficult to think of illustrations of such a state of matters. Suppose we have a speaking tube, with a joint of thin metal at one part which can vibrate like the plate of a telephone. The more easily this is set in vibration, the more will the sound waves, set up at the mouth-piece of the tube, be weakened in passing the joint; the more will the conductivity of the tube, we may say, be diminished here. But it will be easier to set up sound waves in the tube at the joint itself, the more vibratile the latter is. In other words, the "excitability" of the arrangement at the joint will be increased as the "conductivity" is decreased. If, instead of the joint, we suppose a number of holes at one part of the tube, the same thing will happen.

Or imagine that we have two boxes of equal dimensions with transparent sides. Put into each an equal weight of metallic sodium. Heat one till it is filled with sodium vapour at a temperature below that at which it would emit light. A beam of sodium light will be weakened, or, it may be, extinguished in passing through the vapour. In this box we may say the "conductivity" for the sodium light is diminished. It will require, however, less heat to make the vapour luminous in this box than to volatilise the sodium in the other, and then raise the temperature to the same point. The "impulse," that is to say, can be more easily evoked where the "conductivity" is lessened. In other words "excitability" is increased by what diminishes the conductivity.

Again, to put the case a little more generally, let us suppose that the molecules of the axis cylinder transmit the nerve impulse only by vibration in the longitudinal direction. Only the component resolved in this direction of the force acting on any given molecule will be effective for the transmission of the wave. In the normal uninjured nerve we may assume that any transverse component is small, because there is no great change in the intensity of the excitation as it passes. Further, let us suppose, that in the polarised nerve vibrations in all directions are rendered easier around the cathode, the molecules being more mobile. In this region the nerve will be more easily thrown into the active state, that is, the excitability will be increased. But an impulse set up at another part will tend to waste itself in the cathodic region, by setting up those irregular vibrations which are not effective for its propagation. The conductivity will, therefore, be diminished here.

It is hardly necessary to repeat that these are only illustrations of what may happen, and are not intended to represent what does happen.

Fig. 13 shews by means of curves how the stimulation effects in the various regions are related to each other, when the current density is taken as abscissa and the galvanometer deflection as ordinate, the stimulus being supposed constant. The continuous curves represent the effect during the flow; the dotted curves, that after opening. In (2) and (3) *e* means extrapolar, *i*, intrapolar stimulation (median). In (1) stimulation is extrapolar. *a* means stimulation on the side of the anode, *c* on the side of the cathode.

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NOTE.—The fact that no rise of temperature can be found in the active nerves either of frogs as shewn by many observers, or of rabbits as I have lately found by a bolometric arrangement* in opposition to Schiff's thermopile observations, is in favour of the view that the vibrations of the molecules of the axis cylinder are specialized for *transmission* of the impulse, and not wasted in the irregular motion of heat vibrations.

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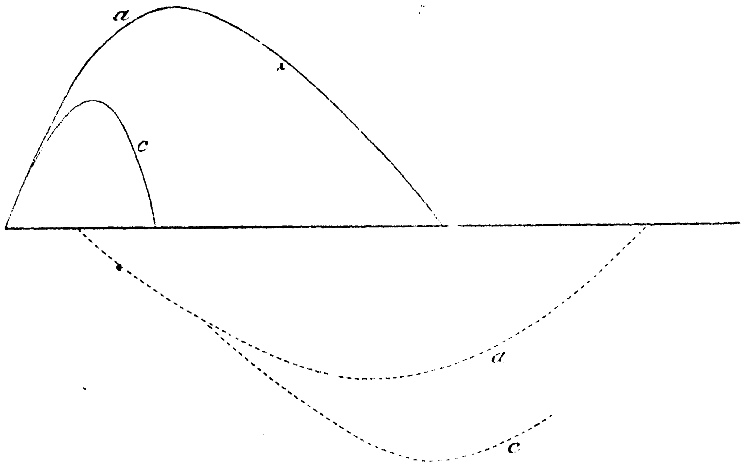
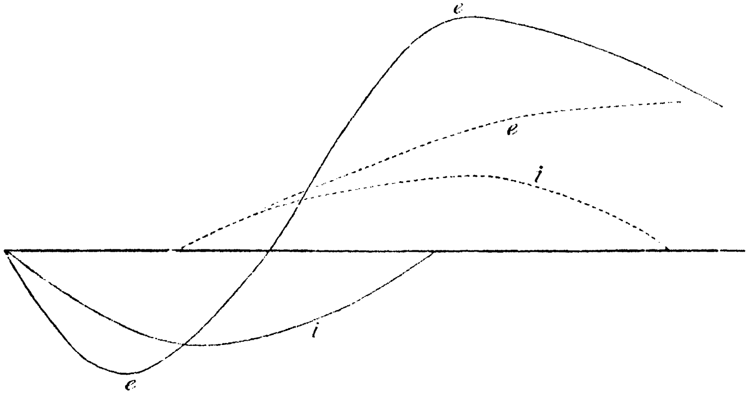
* My experiments are not yet complete, and I intend to repeat them with a more sensitive apparatus, and to extend them to the spinal cord and the grey matter of the brain.

Fig. 13.

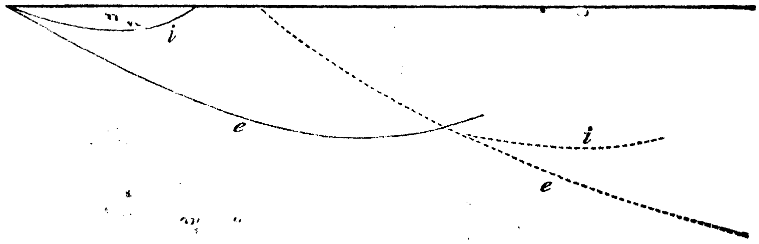
(1) Intrapolar.



(2) Extrapolar anodic.



(3) Extrapolar cathodic.



On the Conditions which affect the Loss of Heat by Radiation from the Animal Body.*

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THE loss of heat by radiation per unit of surface of a body in an enclosure of constant temperature depends upon two factors, (1) the nature of the surface, and (2) the excess of temperature of the surface over that of the enclosure. It is only, however, in vacuo that a body is ever cooled entirely by radiation. When the enclosure contains gas there is always surface conduction. When the excess of temperature is small, the loss of heat varies approximately as the product of this excess into a constant, the coefficient of emission or emissive power, which depends upon the nature of the radiating surface. In this, the simplest, case, we have

$$-\frac{d\theta}{dt} = c\theta \qquad (1)$$

as the expression for the rate of cooling, where θ is the excess of temperature, and c a constant.

Dulong and Petit have given the following formula, which agrees fairly well with the observed facts for a wide range of temperature:

$$-\frac{d\theta}{dt} = c(a^{\tau+\theta} - a^{\tau}) \qquad (2)$$

where θ is as before the excess of temperature of the radiating body, τ the temperature of the enclosure, c a constant depending on the nature of the body, and a an absolute constant which for the centigrade scale is 1·0077. Various other formulæ have been deduced. In all of them the two factors mentioned above are of course involved; and it will be sufficient for our purpose to observe that for such differences of temperature as exist under normal circumstances between the skin and the air of a room, the rate of cooling of a thermometer or a small metallic ball is somewhat greater than that given by formula (1)—for a difference of temperature of 20°, not more than 6·5 per cent greater.

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* This paper is intended to sketch an experimental method and to outline a discussion rather than to give a sustained and detailed account of results. A few of the measurements have been given, however, as illustrations.

When we come to the problem of radiation from the skin of an animal, we find the difficulties which attend the subject even in the case of non-living matter much increased. We have no longer a radiating surface of definite and constant character. Not only may the difference of temperature between the skin and the environment change from time to time, but the physical condition of the epidermis itself may undergo variations, independent of or consequent upon, variations in the state of the corium. The factor c in equations (1) and (2) can no longer be assumed to be constant. Apparently from Masje's results (Virchow's *Archiv*, Bd. 107, pp. 17—71 and 267—290), the change in the emissive power of the skin may be so great, under certain conditions, as to mask and even to annul and reverse the effect of variations in the temperature. These conditions are, however, to a great extent abnormal. But in considering the part which radiation plays in the normal heat loss, we are met by the question, *What is the normal radiating surface in man and animals?* Is it really the naked epidermis? A little confusion on this point seems to exist in the minds even of some physiologists. Certain it is, that the enormous proportion of the heat lost by radiation to the total heat loss, which is observed when the skin is exposed in a physiological experiment, is not a normal ratio. Even in the case of the lower animals it can exist only under highly artificial conditions. In the first place only a small proportion of the naked epidermis is exposed under normal circumstances, and under the conditions which favour a rapid radiation. In man cooling by radiation is practically confined, outside the tropics, and in civilized races, to radiation from the clothes. In warm-blooded animals it is confined to radiation from hair or feathers. In no case can the coefficient of emission of the real radiating surface alter much. It is again a non-living surface, whose properties may indeed be altered by physical changes in the air, but the radiation from which must be chiefly affected by changes in the temperature-difference between it and its surroundings.

But secondly, it is only when the body is at rest in still air that radiation can play a great part even in cooling the covering of the skin. When either the body or the air is in motion much of the heat lost must be given off by conduction and convection. Even a body with lamp-blacked surface, cooling in a closed space with lamp-blacked boundaries, loses only about half its heat by radiation, the other half being carried away by the air.

In normal circumstances, then, radiation is not the chief way in which the body loses heat. Further, what radiation there is is chiefly from surfaces whose excess of temperature is maintained by conduction from the epidermis, and the emissive power of which cannot alter much. A regulation of the heat loss by alterations in the amount of radiation cannot take place to any great extent by changes in the emissive power of the epidermis, cannot probably take place at all by alterations in the emissive power even of the physiological protective coverings. If such a regulation exists, it must be by means of changes in the conducting power of the epidermis, and of the temperature of the radiating surface.

This paper includes (1) observations on the temperature of the skin of man when covered and exposed, and on the temperature of the surface of physiological and artificial protective coverings in man and one or two animals; (2) measurements of the amount of radiation from the normal radiating surfaces, and from skin which is normally covered; and (3) simultaneous measurements of temperature and radiation.

Methods and instruments used.

The relation between the temperature and the resistance of a metallic conductor gives us the means of constructing thermometers and radiometers of great accuracy and sensitiveness, and of late years the principle has been more and more applied in physical investigations in the domain of heat. It has also been introduced into physiology. Masje in 1887, in an elaborate research on radiation from the human skin, of which we shall have more to say later on, used a grating of tinfoil as radiometer; and quite lately Rolleston has attempted, with a negative result, to detect an increase of temperature in active nerves by means of a resistance thermometer of platinum wire. (*Journal of Physiology*, XI., 208-225.) More than three years ago I made a similar attempt with a grating of gold leaf, prepared by fastening the leaf upon a large coverslip, and then cutting the grating out, the whole being covered with a thin layer of varnish. Six sciatic nerves of the frog were arranged on one grating and covered with another, the two being connected in series, and balanced by a similar pair. The result of the first experiments was, like Rolleston's, negative, but the work had to be broken off before enough had been done to warrant a definite conclusion, and it was never resumed. Before the gold leaf had been tried an attempt was made to produce a grating on glass by the process of electro-gilding, with fair success; but it was feared that the plumbago lines upon which the gold was deposited might affect the accuracy of the apparatus, and it was not used. In this work I have employed gratings of lead paper.

I. MEASUREMENT OF THE TEMPERATURE OF THE RADIATING SURFACES.

I do not know of any investigation of the temperature of the skin which has been made before with an apparatus of this kind; yet the method marks itself out as pre-eminently suitable for the measurement of the temperature of surfaces. What is required here is an instrument which can be accurately adjusted to the surface, which will quickly and certainly acquire the same temperature, and which at the same time will not sensibly alter that temperature by preventing the free radiation and conduction of heat. The resistance thermometer fulfils the first condition because it is easy to give it any form. It fulfils the second, because its capacity for heat can be made extremely small by using very thin wire or foil, by which at the same time its sensitiveness is increased. It fulfils the third condition, both on account of its small capacity, and because it can be so arranged that the average temperature of an area can be found without covering more than a portion of it. It has great advantages over the thermopile because of its small mass, its plasticity of form, the quickness with which it comes to its position of equilibrium, and the fact that the resistance is approximately proportional to the temperature. Its greater sensitiveness is an advantage for some purposes, but not for measuring the temperature of the skin. It is of course no disadvantage, because it can be easily reduced. All this is true of almost any form of resistance thermometer. Another advantage, for work where extreme accuracy is not required, is that anybody who can measure a resistance can make himself a sensitive thermometer in a very short time out of the commonest materials. I used three forms in measuring the temperature of the skin. In making the first, a piece of ordinary lead paper was fastened to a coverslip with shellac varnish. It was then cut out into a grating, with a sharp pointed knife, each bar being about a millimeter in breadth, and the distance between two adjacent bars being about half a millimetre. The coverslip was fastened to a simple wooden holder, the grating covered with a slip of the thinnest glass, and the unscored ends of the lead paper were turned up round the holder, and tacked to it by a small copper nail on each side, which was soldered to copper connections. The resistance was between three and four ohms and the area of the sensitive surface a little more than a square centimetre. It would be perfectly easy to make the area very much less than this, but what I wanted to measure was not the very small differences of temperature which can exist in neighbouring portions of the skin, but the average temperature of areas of moderate size.

This grating was balanced by another of precisely similar construction, the two forming the arms of a Wheatstone's bridge, which was completed by a graduated wire with a sliding contact.

Two methods of observation were used. In the first the bridge was balanced, one of the gratings applied to the skin (the same one was always employed), and the resulting deflection read off. This is proportional to the difference of temperature between the two gratings, and by an experimental graduation the value of the deflection in degrees is obtained, this value being the excess of temperature of the skin over that of the air. In the other method two equal resistances were introduced one at each end of the bridge wire. The resistances were of such amount that with the maximum difference of temperature between the gratings the slider had to be moved over three-quarters of the length of the wire in order to restore the balance. Instead of reading the deflection, the position of the slider with null deflection was read before and after the application of the grating to the skin. The experimental graduation gave the difference of temperature corresponding to a given difference of position of the slider. Experiments 1 and 2 are examples of the two methods. In using the first method it was necessary to be careful that the battery was always set up in the same way, so as to have a constant E.M.F. and resistance.

EXPERIMENT 1. Deflection read { Δ = difference of temperature between the part and the air of the room.
T = temperature of the part.

Region.	Deflection.	Δ	T	Temperature of room.
Anterior surface of left forearm.	222	16·8	34·4	17·6
Posterior surface of left forearm.	218	16·4	34·0	17·6
Anterior surface of left arm over belly of biceps.	232	17·6	35·0	17·4
Left leg over head of tibia.	190	14·4	34·9	17·5
Skin just below xiphoid cartilage.	226	17·2	34·7	17·5
Skin over sternum.	206	15·6	33·2	17·6
Trousers over anterior surface of left thigh (nothing between trousers and skin).	82	6·1	23·7	17·6

EXPERIMENT 2. Position of the slider read.

Region.	A	T	Temperature of room.
Palm of left hand.	12·58	30·95	18·37
Forehead.	14·70	33·04	18·34
Right cheek.	14·92	33·21	18·29
Left breast.	16·01	34·40	18·39
Right hypogastrium.	17·00	35·16	18·16
Over apex beat.	16·32	34·57	18·25
Sole of left foot.	12·75	31·05	18·30

The second form of grating was designed to allow part of the area whose temperature was being measured to be still in free communication with the air, or perhaps more correctly to measure the average temperature of alternate strips of the given area. A lead paper grating was cut out on thin cardboard, the latter being cut clean away in the intervals between the bars of the grating. The breadth of an interval was the same as that of a bar. The surface was lightly varnished, and the whole mounted in a handle attached to one side. Experiment 3 is an example of the results with this grating.

EXPERIMENT 3. The measurements were made immediately after those of Experiment 2, on the same parts, and as far as possible under the same conditions.

Region.	A	T	Temperature of room.
Palm of left hand.	12·83	30·99	18·16
Forehead.	14·41	32·71	18·30
Right cheek.	14·63	33·07	18·44
Left breast.	15·93	34·18	18·25
Right hypogastrium.	16·82	35·07	18·25
Over apex beat.	16·68	34·68	18·00
Sole of left foot.	12·50	30·61	18·11

A third form of lead paper grating was devised to allow the outer surface of the metal to be freely exposed to the air. The grating was attached to a narrow frame of cardboard, and supported by a wide-meshed silk netting on its outer surface, while the surface next the skin was varnished. With care it was possible to get steady contact without injuring the grating. Experiment 4 is an example. The measurements were made immediately after those of the last experiment.

EXPERIMENT 4.

Region.	Δ	T	Temperature of room.
Palm of left hand.	12·44	30·72	18·28
Forehead.	14·14	32·60	18·46
Right cheek.	14·87	33·00	18·13
Left breast.	15·91	34·01	18·10
Right hypogastrium.	16·56	34·83	18·27
Over apex beat.	16·12	34·42	18·30
Sole of left foot.	13·00	30·84	17·84

In each experiment the temperature of the parts usually covered by the clothes was taken immediately after exposure, and the results of such measurements are fairly uniform. Experiments 3 and 4 shew temperatures which do not differ much from those of Experiments 1 and 2; but on the whole the temperatures are somewhat lower in the former. This was found to depend not upon cooling due to previous esposure, but upon the kind of instrument used. The first form described reads a little too high, probably because the free radiation and conduction of heat is interfered with, and the evaporation of sweat checked, more than when the other forms are used. Still it is fairly accurate and suitable for every purpose of relative temperature measurement, although not so suitable as the others when the actual temperature of a part has to be determined to the second decimal.

But almost any form of resistance thermometer would be a great improvement upon the mercury thermometer for measuring skin temperatures. The great constancy of the readings for parts of the skin where the temperature has reached its stationary state, *e.g.* the hands or face of a man who has sat for some time at rest in still air, or a part of the usually covered skin which has cooled to its minimum, is a good proof of the reliability of the method.

When a part of the body which is normally covered is exposed to the air of a cool room the temperature declines progressively to a minimum, which, with small variations, it maintains. Experiments **5** and 6 are Examples.

EXPERIMENT 5.

	Time.	Δ	T	Temperatur of room.
Anterior surface of left forearm exposed at 4.10...	4.10	15·23	33·43	18·20
	4.17	14·81	33·23	18·42
	4.23	14·10	32·66	18·56
	4.29	13·77	32·37	18·60
	4.37	13·11	31·71	18·60
	4.42	13·40	32·02	18·62
	4.55	13·40	32·02	18·62

The temperature remained constant at about 32·0 for 20 minutes longer, when the experiment was broken off.

EXPERIMENT 6.

	Time.	Δ	T	Temperature of room.
Anterior surface of left forearm exposed at 5.5 ...	5.1	—	—	—
	5.5	11·82	32·43	20·61
	5.15	11·04	31·64	20·60
	5.24	9·40	30·03	20·63
	5.83	9·65	30·25	20·60
	5.40	9·42	30·02	20·60

The cooling is not uniform over the whole of the exposed surface. In general the extensor surfaces of the limbs cool more quickly than the flexor, but sometimes there may be little difference. The decline of temperature is especially marked where there is no great thickness of muscle between the skin and the bone, or where there are only tendons. The skin over the patella and shin cool rapidly, while the temperature of the back of the hand is nearly always less than that of the palm.

The temperature of the surface of the clothes in man and of the hair of animals is a very important element in determining the amount of radiation. It depends chiefly upon the temperature of the epidermis, the conductivity of the coverings, and the temperature of the air. It must be remembered that air when not free to move is a very bad conductor of heat, and the air between the clothes and the skin and in the pores of the clothes, and between the hairs and feathers of animals, is a most important protective covering. The aqueous vapour in the air also absorbs radiant heat from a low temperature source with great readiness, as Tyndall has shewn (*Phil. Trans.* 1861); and still air near the skin must be saturated or nearly saturated.

The difference of temperature between the surface of the clothes and the air will determine the amount of radiation and conduction, and it is certain that in man a great part of the heat regulation consists in keeping this difference approximately constant. If the temperature of the air falls, that of the surface must fall too, unless the loss of heat is to increase. Between the surface of the clothed skin and the outer surface of the clothes the slope of temperature must become steeper. Now the flow of heat by conduction is proportional to the slope of temperature and the specific conductivity, and inversely proportional to the thickness of the conductor. The flow must be quickened when the slope of temperature becomes steeper, the other factors remaining unaltered. But if the thickness of the conductor be increased or its specific conductivity diminished, the flow may be kept the same as before. This is, of course, what happens when additional clothes, or clothes of a warmer kind are put on in cold weather. When this compensation is not complete, the slope of temperature may be made less steep by warming the air artificially. When from any cause compensation cannot be obtained by variations in the factors governing the outflow of heat, the increased flow may be balanced by an increased production, due either to visible muscular contractions, voluntary or involuntary, or to increased metabolism unaccompanied by such contractions. Heat regulation by alteration in the heat production seems to be more complete in the lower animals than in man (Loewy, Pflügers *Archiv,* Bd. 45, S. 625). This is what we should expect, not only on account of the smaller size of the animals generally used for experiment, but because animals of any size have very little voluntary control over the difference of temperature of the radiating surface and the surroundings.

I have measured the temperature of the real radiating surfaces in a man clothed in the ordinary way and seated in a still atmosphere, and at the same time measured the radiation. The temperature of the room varied from 17° C. to a little over 20° C. The excess of temperature of the surface of the clothes varied from 6°·1 over the thigh, which was covered only by the trousers, to 1°·51 outside of the coat when it was buttoned up. Outside of the waistcoat it was in one experiment 4°·17. Between the waistcoat and the skin there was a linen shirt and a flannel undershirt. The coat sleeve over the fore-arm had an excess of 4°·32; over the upper-arm, of 3°·88. The excess of temperature did not vary much for a change of a few degrees in the temperature of the room.

The rest of the radiating surface, the skin of the hands, neck, and face, and the hair, had of course a much greater excess of temperature, ranging from 11°·61 on the palm and 10°·83 on the dorsum of the hand to 14°·54 at the side of the neck, and 13°·86 on the cheek, the surface of the hair having an excess of 7°·4.

The skin of a guinea-pig at one place had a temperature of 34°·50, the surface of the hair over it a temperature of only 29°·11; the skin at another place 35°·83, surface of hair, 29°·56; the skin of a rabbit, 36°·80, hair over it, 31°·54.

II. MEASUREMENT OF THE QUANTITY OF HEAT RADIATED.

Arrangement. I used at first a thermopile, but soon abandoned it for a lead paper grating fastened on a frame of stout cardboard, and blackened on one surface. It was balanced in the Wheatstone's bridge by a similar grating, and the measurements were taken in the manner described for the temperature. The grating which received the radiation was of course kept at a constant distance (4½ centimetres) from the radiating surface.

The amount of radiation varied greatly at different parts of the surface. Over the clothes it was nearly proportional to the excess of temperature, as was to be expected, the outside clothes being all of the same material. The radiation from the exposed skin was also approximately proportional to the excess of temperature, but in a higher ratio, the skin having a greater coefficient of emission than the clothes (these were of light grey material). The total radiation under the most favourable circumstances did not exceed the rate of 700,000 calories in 24 hours for a body-weight of 70 kilogrammes. Taking the surface and, therefore, the radiation as proportional to the body-weight, this would give 820,000 calories for a bodyweight of 82 kilogrammes. Helmholtz has calculated the total heat loss from the skin, by evaporation, radiation, and conduction, for a man of 82 kilogrammes weight, at about 2,180,000 calories for a temperature of the air of 20°. If we substract 280,000 calories for evaporation, we get about 1,800,000 calories as the loss by radiation and conduction together; and of this the radiation would account for less than half.

Masje calculated from his results, that the quantity of heat radiated from the body of a man of 82 kilogrammes weight was 1,728,000 calories, which agrees, according to him, almost precisely with the calculated heat loss by the skin, exclusive of that due to evaporation. This leaves scarcely anything for conduction, which is certainly a mistake. It is beside the point to say that "the conductivity of still air is very small, almost 20,000 times smaller than that of copper." Under the conditions of his experiments and indeed under any circumstances in which the skin is exposed to the air of a room, conduction and convection must play a great part. The reason why Masje got such a high value for the radiation is not because the other portions of the heat loss were very small, but because the total heat loss in his experiments was very great, much greater than the normal. The agreement of his calculation for radiation alone with Helmholtz's calculation for radiation and conduction together is only accidental. What Masje measured was the heat radiated from the *naked skin* in a cool room; and nobody can doubt that the total heat loss, with an air temperature of 10°—15° C. must be greater when the body is naked than when it is clothed. This is true, not only when the whole body is actually stripped, but also when the heat loss is reckoned from that of a number of limited areas, singly and successively exposed. If Masje had measured the total heat loss by radiation and conduction, under the conditions of his experiments, he would have found that the fancied agreement disappeared, and the disappearance of it would be a necessary proof of the correctness of his results. The mistake, however, can only be considered as a slip in a very thorough and scientific paper; but it is well to point out that Masje's whole investigation, interesting as it is, has only a limited application to the question of heat radiation from the body under normal conditions.

For example, in one of my experiments the heat radiated from unit area of the palm of the hand was to that radiated from the surface of the sleeve of a thin flannel shirt over the anterior surface of the corresponding forearm, as 204 to 42, or in round numbers 5 to 1. The radiation from the naked skin of the anterior surface of the forearm was to that from the same thin covering as 270 to 42, or more than 6 to 1, while its proportion to the radiation from the surface of the coat was as 270 to 28, or nearly 10 to 1. From the cheek the radiation was almost exactly six times as great as from the flannel covering the forearm. It is evident that if we were to take the radiation from the naked skin as the measure of the normal heat loss by radiation, the value would be far too high; for the clothes are warmed chiefly by conduction.

We have seen that there are two factors which may affect the amount of radiation, the temperature difference and the emissive power. Masje could not find any decided influence of the former on the radiation from naked skin; but he found that the emissive power varied in a very remarkable manner. He found that when the whole body or a large part of it was exposed to the air of a cool room, the radiation increased with the time of exposure; and when the temperature of the room was only 9°—10° C., it might in less than an hour reach double or even quadruple its initial amount. He ascribes this to changes, perhaps partly produced reflexly through nervous influence, which alter the emissive power of the surface. I do not dispute the accuracy of the observations on which he rests his conclusions, although I am unable from my own experiments to confirm them, as I worked with a higher temperature of the air. It is to be expected that physical changes in the radiating surface, such as must take place in the skin both from physical and physiological causes, affecting notably the amount of moisture contained in its superficial layers and on its surface, should affect also the emissive power. And although the outer layer of the epidermis can scarcely be susceptible to direct nervous influence, yet it is not impossible that changes in the rete Malpighii brought about through efferent nerves acting directly on its cells may have the supposed effect. On the other hand there are so many known factors by which the amount of radiation, as measured by a pile or a bolometer, may be influenced, that it is only when these are obviously insufficient to explain a phenomenon, or when there is strong direct evidence that they are not connected with it, that we should call in the aid of "direct nervous action."

There is one factor which must affect all radiation experiments on the animal body, and especially on the human skin; and that is the quantity of sweat given off. Apart from its effect on the temperature of the skin, which will influence the quantity of heat emitted, the amount of watery vapour, and perhaps of other substances such as the aromatic bodies in sweat, in the layer of air next the skin, will affect the proportion of the radiated heat which reaches the recording instrument. The absorptive power of water vapour for radiant heat from a source at the temperature of the surface of the body is very great. Carbonic acid gas is also a very much better absorber than dry air. Certain organic vapours in very minute traces almost prevent radiant heat from passing. In still air there must be a layer next the skin which contains more watery vapour than the atmosphere. When the sweat glands are active this layer will be of greater thickness and more highly charged with watery vapour, carbonic acid, and possibly with other substances which are even better absorbers of radiant heat, than when the secretion of sweat is slow or in abeyance.

If we take the quantity of water given off as insensible perspiration for an average adult, at, say, 650 c.c., it is easy to calculate the volume of dry air which would be saturated by it for any given temperature. One gramme of aqueous vapour can saturate, in round numbers, 33,000 c.c. of dry air at 30° C., and 650 grammes would saturate 21,450,000 c.c. Taking the surface of the body at 20,000 sq. cm., we get about 1,070 c.c. of air saturated at 30° C. per centimeter of surface in the 24 hours. This is equal to about ¾ of a c.c. per minute. In a minute the evaporation from the skin would suffice to surround the body with a shell of air, saturated for the temperature of 30° C., of three-quarters of a centimetre in thickness, if there was no movement of the air.

Séguin's estimate of the material given off by the skin at $\frac{1}{67}$ of the bodyweight in 24 hours would correspond to about double this thickness of saturated air at 30° C.; and to a saturated layer 3 centimeters thick at 20° C. For originally dry air at 10° C. the layer would be rather less than 5 c. in thickness; and for air originally half saturated at 10° C., the thickness of the layer raised to saturation point in a minute would be 10 c.

Of course we cannot assume that saturation does actually go on in still air so quickly as this. But if we consider that the average rate of evaporation for the 24 hours over the whole surface may be much exceeded at certain times and for particular parts of the skin; and further that aqueous vapour does not necessarily diffuse away at the rate depending on its density but may be condensed on dust particles in the air and re-evaporated, we shall see that some absorption of the heat radiated from the body must take place between the instrument and the skin, and that this will be greater the more freely the sweat glands are acting.

A small apparent increase in the radiation when the skin is exposed for some time in a room, with a temperature of 17° C. to 20° C. might be explained as due to diminished activity of the sweat glands, and therefore to diminished absorption. But the increase observed by Masje with a temperature of the air of 10° C. to 13° C. is too great to be thus accounted for, and the initial intensity of radiation does not seem to leave room for a very extensive absorption. Besides, a *warm* bath seemed in his experiments to cause an increase of radiation, even after the skin temperature had sunk to normal. I cannot say that I have been able to satisfy myself that the increase of radiation which undoubtedly follows the use of a bath a few degrees higher than the skin is due to anything else than the increase of temperature of the radiating surface. But this may again be due to the temperature of the room being higher than in Masje's experiments.

The effect of some antipyretics in increasing radiation certainly seems to favour the view, that the emissive power is increased; but when an antipyretic causes flushing of the skin it must increase the *temperature* of the radiating surface. Antipyrin, as Masje rightly remarks, reduces the temperature in the axilla, at the same time that it increases the radiation from the skin. But reduction in the temperature of the axilla or in the temperature of the blood, is not the same as reduction of the temperature of the naked skin. The radiation from a flushed skin must come partly from a layer as deep as the most superficial bloodvessels, else we should not see the flush; and doubtless rays of greater wavelength than the extreme visible red can pass through the epidermis.

The greatest difficulty in the way of the explanation of such immense changes in the intensity of radiation as Masje saw, by changes in the emissive power, is their very magnitude. The emissive power of the skin under *normal* conditions is high. I have found that a thin layer of lampblack does not much increase the radiation from the palm. In some experiments it even seemed to diminish it. But there is a possible fallacy here. Some of the radiation from the skin certainly comes from the deeper layers. The lampblack will absorb this, and being at a lower temperature at first it will not give it all out again. But dead skin is also a good radiator. Two equal cubes of thin metal were covered with human skin. The skin covering one of them was coated with lampblack on the outside. That covering the other received a similar coating on the inside before being put on, so that the conductivity might be the same in both. The cubes were filled with water at 40° C., and allowed to cool. The rate of cooling showed that the lampblacked surface did not radiate much faster than the skin. But if the emissive power of skin can be increased fourfold, it must before the increase be less than one-fourth that of lampblack. Masje found that when the skin of the arm was cooled 3° C. to 4° C. below the normal the radiation fell off instead of increasing, and he explains this as due to the effect of the diminished temperature more than counterbalancing the effect of the increased emissive power.

It is difficult to see why if the emissive power can be increased fourfold by cooling the surface, a decline of 3° C. in the surface temperature should have any sensible effect in checking the radiation. From equation (1), p. 101, we see that the radiation will remain constant if c is increased in the same proportion as θ is diminished. Taking 3°—4° as the utmost by which the temperature of the skin can be diminished without diminishing the radiation, we find that an increase of one-fifth in the coefficient of emission would in Masje's experiments be enough to balance the fall of temperature. But if an increase of fourfold is possible, why is an increase of a fifth not forthcoming?

If we take equation (2), p. 101, we get for the radiation from skin at 31° C., with a temperature of the room of 15° C., the expression

$$R = c(a^{31} - a^{15}) = ca^{15}(a^{16} - 1),$$

and for the radiation from the skin at 28° C.,

$$R' = c(a^{28} - a^{15}) = ca^{15}(a^{13} - 1) \therefore \frac{R}{R'} = \frac{a^{16} - 1}{a^{13} - 1}.$$

Now $$a = 1{\cdot}0077, \text{ and } \frac{R}{R'} = \frac{1306}{1049}, \text{ or about } \frac{5}{4}.$$

If we take 4° as the fall of temperature,

$$\frac{R'}{R'} = \frac{1306}{964}, \text{ or about } \frac{4}{3}.$$

So far as the temperature factor is concerned the radiation would only be diminished by $\frac{1}{6}$ for a diminution of 3° C.; and by $\frac{1}{4}$ for a diminution of 4° C.; again we are confronted by the question why the increase in the emissive power, which can assume such proportions when the temperature is lowered by 2° C., should be unable to compensate for the trifling diminution of radiation ($\frac{1}{15}$ or $\frac{1}{12}$ at most) caused by a further lowering of 1° C. Either the emissive power cannot alter so much as Masje thinks, or it is not altered in the same sense, or in the same proportion if the sense be the same, for a fall of temperature of the skin of 3° C. as for a fall of 2° C. It will not do to say that it reaches a maximum when the skin is cooled, say 2° C. It must decline from this maximum or possibly sink even below its original amount, if Masje's explanation is to be held sufficient. The greater the changes in the emissive power which must be postulated in order to explain his results with moderate cooling, the more difficult does it become to give a consistent explanation of what happens when the cooling is carried a little farther. Even if we suppose that the greater part of the radiation is from layers below the surface, and that the absorptive power of the superficial layers for this radiation is the chief variable, the difficulty is still to explain how so large a variation can be brought about, and why it should so abruptly change its sign. With an external temperature of from 17° C. to 20° C. I have certainly found that the temperature of the skin is a far more influential variable than the emissive power. Masje, on the other hand, working with lower external temperatures, found just the reverse. No entirely satisfactory explanation occurs to me of this difference—discrepancy it can hardly be called, because there is no reason why the factors which determine the amount of radiation should affect it in the same way with a low, as with a moderately high external temperature. The influence of the temperature factor is very clearly seen in parts of the skin which are normally exposed, while the emissive power of such parts remains approximately constant. Experiment 7 shews the effect of various conditions of such a surface and of changes of its temperature on the radiation. When the temperature of the hand is raised or lowered by immersion *for a short time* in a bath, the radiation is correspondingly increased or diminished. This contrasts with the behaviour of the normally covered skin as observed by Masje, who found that cold baths might greatly increase the radiation.

EXPERIMENT 7. Palm of left hand.

Time.	Condition of Surface.	Deflection.	
11.30 a.m.	Moist with sweat.	100	The temperature of the room during the observations varied from 20° C. to 20°·3.
11.32 „	Dried lightly with cloth.	130	
11.36 „	Washed in water at 20° and dried.	122	
12.30 p.m.	Felt cool.	98	
2.30 „	Immediately after walking—felt warm.	121	
2.40 „		126	
2.45 „	Covered with vaselin.	86	
2.50 „	Still covered with vaselin.	78	
2.55 „	Vaselin washed off—hand dried without rubbing.	126	
2.59 „	Palm covered with lamp-black.	101	
3.2 „	„ „	105	
3.4 „	„ „	112	
3.6 „	„ „	98	
3.9 „	Lamp-black removed.	120	
3.10 „	„ „	124	
3.15 „	Rubbed briskly with towel—felt very hot.	158	At 3.18 the radiation had sunk to 123.
3.20 „	Heated in dry air at 50° C. for 1 minute.	148	
3.25 „	After being 1 minute in bath at 19° C.; dried without rubbing.	96	
3.30 „		86	
3.35 „	After being 1 minute in bath at 27° C.	99	
3.40 „	After being 2 minutes in bath at 36° C.	134	
3.50 „	After being 2 minutes in bath at 46° C.	168	At 3.45 radiation was 125.

A division of the scale represents about ·000006 small calories (gramme-degrees) per second per c.c. of surface.

III.—SIMULTANEOUS MEASUREMENT OF THE SURFACE TEMPERATURE AND THE RADIATION.

I do not propose to do more here than to describe the method of making the observations and to give a single example of them, as I hope to have an early opportunity of returning to the subject in connection with some calorimetrical work.

Both the temperature and the radiation were measured as before by the resistance method, and the radiometer was the same lead paper grating as was previously used. The thermometer applied to the skin consisted of a narrow strip of varnished lead paper, forming the sides of a square whose internal area was equal to that of the sensitive part of the radiometer. It was attached to the radiometer at a distance of 4½ centimetres; so that when a part of the surface of the body was applied to the thermometer it was in the proper position for the measurement of the radiation. The instruments, balanced by two precisely similar arrangements, were each introduced into a Wheatstone's bridge, the two systems being quite separate and the galvanometers independent. The deflections were simultaneously read. Strictly speaking it was not the temperature of the radiating surface itself which was measured, but that of its boundary, which must be approximately the same when the area is not too large.

Experiment 8. Palm of right hand.

Condition.	Radiation.	Δ	T	Temperature of room.
Normal.	107	10·40	31·0	20·60
Arm held above head for 2′.	70	9·05	29·65	
Hand hanging down for 2′.	128	11·1	31·7	
Hand kept at level of heart for 2′.	120	10·4	31·0	
Band round wrist so as to prevent the venous return.	121	10·9	31·4	20·5
Hand immediately after bath at 18° for 2′.	90	9.50	30·11	20·61
Hand after brisk rubbing.	152	14·07	34·57	20·50
Hand after bath at 33° for 2′.	112	10·84	31·34	20·50
Hand after bath at 45° for 2′.	174	15·10	35·52	20·42

Δ means, as before, the excess of temperature of the skin over the air.
T the temperature of the skin.

It will be seen that the radiation from the palm is in most cases approximately proportional to the temperature; and therefore the coefficient of emission for the palm may be looked upon as fairly constant. This does not apparently apply to the normally covered skin, nor to the skin in fever. Masje states that in fever the radiation is less than the normal, although the temperature of the skin is greater. This can scarcely be true of the whole heat loss by the skin, for, in fever, after the stationary temperature has been reached, the loss of heat must equal the production, and the production is increased. More heat must therefore be lost in other ways than is normal; by conduction and convection and by respiration that is to say.

The general conclusions to which I have been led are that under normal circumstances the heat loss cannot be regulated by alterations in the emissive power of the radiating surface either in man or animals, and that, for the external temperatures with which I have worked, no marked change in the emissive power of the human skin can be brought about by heating or cooling it. The difference of temperature between the radiating surface and the environment is therefore the chief factor which affects the heat loss. When this is increased the heat loss is in general increased, whether the greater difference of temperature be brought about by lowering the external temperature, or by increasing that of the radiating surface, or by substituting for the normal radiating surface another of higher temperature, or greater emissive power, or both.

When a large part of the skin of an animal which is normally protected by an artificial or natural covering is exposed to air at a temperature of 15° C. to 20° C., the loss of heat by the skin is increased; and it depends (*a*) upon the size of the body, (*b*) upon the perfection of the heat-regulating mechanism, whether the internal temperature falls or not. The larger the body the less must the proportional increase of the heat production be, in order that the internal temperature may remain constant.

In small animals, such as rabbits and guinea-pigs, it is astonishing how little an increased heat production can supply the loss of a considerable part of the protective covering, or make up for an alteration in it which increases the outflow of heat. It has long been known that a rabbit dies when its skin is varnished.

The superstition still lingers in some text-books that this is due to interference with the excretory functions of the skin. This explanation may perhaps hold good in the case of animals like the horse. It is not necessary for the case of the rabbit and the guinea-pig. For, without varnishing at all, if the hair be removed from the greater part of the body of either of these animals by rather close clipping or still better by shaving, and the animal be kept in an empty box so that it cannot cover itself, it dies in summer weather with an air temperature of 15° C. to 18° C., and that sometimes in 20 to 30 hours. The rectal temperature sinks fast, notwithstanding almost constant involuntary muscular contractions. If the radiation from the skin be measured, it is found to be far in excess of that from the hair. The animal is evidently losing heat more rapidly than it can produce it. The heat regulating mechanism is overmastered by the sudden increase in the heat loss from the skin. If now, before the cooling has gone too far, the animal be placed in a warm chamber, the rectal temperature rises again, and it recovers completely. The increase of heat production can only respond within somewhat narrow limits to an increased heat loss; and it would seem that if the heat production once begins to lag behind the heat loss, if a considerable diminution of the temperature of the blood has actually taken place, it becomes more and more difficult to restore the balance.

When the skin is varnished the same kind of thing goes on. The hair owes its low conducting power chiefly to the large amount of air which it keeps at rest around the animal. When it is varnished over, a great part of this air is expelled, the hair is flattened down on the skin, and the escape of heat is accelerated. The dilatation of the vessels which is said to take place under the varnish would increase this still more.[1] Possibly the emissive power of the varnish may be greater than that of the normal radiating surface. I have not, however, found this the case for the skin of man. At any rate, an alteration in the normal radiating surface, whether produced by varnishing or by removal of the hair may be sufficient to baffle any attempt at making up the increased loss by increased production, and the lower the external temperature the more easily will this take place.

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[1] Any direct or reflex "tonic" effect which the actual contact of the air may have on the skin is of course lost.

Electrolysis of Animal Tissues, with Special Reference to Electro-therapeutics.

BY

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WHEN a current passes through a living tissue it produces two kinds of effects, (1) physiological, (2) chemical or physical. The physiological effects no doubt depend in some cases, if not in all, upon precedent chemical changes, and are accompanied or followed by further changes of the same kind. The more purely chemical and physical changes may lead in their turn to physiological effects. So that no hard and fast line can be drawn between the two classes. But it is easy to lay down the conditions which determine the predominance of the one rather than the other. A current of short duration produces chiefly physiological action. In other words, its effect is mainly to start the activity of the excitable tissues.

An induction shock sufficient to cause a maximal contraction of a muscle produces only a very slight electrolytic decomposition. A galvanic current which is gradually increased from a sub-minimal strength without causing excitation may yet bring about profound chemical effects ending it may be in the death of the tissue. A given quantity of electricity passed in the form of a series of swift shocks may throw an animal into violent tetanus, may exhaust the muscles and nerves, and lead to gradual or sudden death, while the same quantity passed as a continuous current may have scarcely any visible physiological effect. The amount of chemical decomposition caused by the current will be the same in both cases, but the physiological changes may be very different. On the other hand, the continuous current may excite, or at least determine, physiological effects which may last through the whole period of its flow, and even continue long after. The investigation of these effects for strong currents, and particularly the investigation of the chemical and physical changes by which they are accompanied, and of the relation between the two, if there be a relation, is a large inquiry, an inquiry of great and even pressing importance in view of the rapid development of electrolytic treatment both in general surgery and in gynecology. This paper is a small contribution to the knowledge of this wide and somewhat neglected region. I propose to deal here chiefly with the chemical and physical part of the subject, leaving the physiological for a future communication. But wherever there appears to be a connection between the two parts, or even a hint of a connection, I shall try to point it out.

The first question to settle was whether the tissues conduct altogether by electrolysis, or whether a part of the current may not be carried by conductors belonging to the metallic class. It is evident that part of the conduction at least must be electrolytic; for the tissues are polarisable; they can replace the liquids of a voltaic cell; their resistance is diminished by heat. They are bathed with blood and lymph. Everywhere in the mass of a tissue there are inorganic salts in solution or perhaps in combination, chemical or molecular, with the proteid substances. These must necessarily conduct part of the current. A larger problem could therefore be stated in this way: What proportion of the current is carried by electrolytes, and what are the electrolytes? The second part of the problem was first attacked. What are the electrolytes? In particular, are the proteids electrolytes? I attempted to answer this for coagulable proteids (egg-albumin, serum albumin, and myosin) in solution by measuring the resistance at a given temperature before and after coagulation. I found that within the limits of error of the methods used heat coagulation produces no change in the resistance. Of this fact there are two possible explanations. Either the coagulable material conducts so badly that any physical alteration in it causes no appreciable change of resistance in the mixture of salts and proteids, or else the conductivity of the pure coagulable proteid, although of the same order as that of dilute saline solutions, is really unchanged by coagulation. The second hypothesis seemed very unlikely. But we know so little about the state of a coagulated colloid that we cannot assume that it does not conduct electrolytically; and more than one solid electrolyte is already known to science. It is certain that salts can conduct the current through a firm colloid jelly about as well as through water; and if this were to hold also for the colloids themselves, the absence of change of resistance on coagulation would give no clue to the relative conductivity of the proteid and saline constituents of a mixture.

In order to eliminate this possibility I supplemented the coagulation experiments by a series in which the resistance of animal solids and liquids containing proteids was measured first in the normal state, and then after abstraction of the salts and other crystalloids by dialysis or digestion with water.

Resistance Experiments. Before and after coagulation.

In measuring the resistance of electrolytes some means must be taken to eliminate polarisation. Three distinct methods have been chiefly used for doing this: (1) the method of alternating currents introduced by Kohlrausch and Nippoldt (*Pogg. Ann.* CXXXVIII. p. 286, Oct., 1869), (2) Paalzow's method (Berlin *Monatsbericht,* July, 1868), and (3) the electrometer method used by Bouty (*Comptes Rendus* CII., p. 1098, and XCVIII., pp. 140, 362, 797, 908).

I employed both the first and the second methods with some modifications. For the first, two thin glass U-tubes of the same dimensions contained the solution, and formed the arms of a Wheatstone's bridge. The tubes had large cups at the ends in which the electrodes, consisting of platinum disks, whose area was large compared with the cross section of the tubes, were placed. The resistance of the arms of the bridge would of course be approximately the same. The bridge was completed by a thin German silver wire 4 metres long and with a resistance of 84 ohms. This was used instead of a box to avoid self-induction. A telephone connected a slider moving on the wire with the junction of the two tubes. Equal and opposite induced currents being sent through the arrangement, the polarisation of the electrodes would be eliminated with a sufficient frequency of vibration of the automatic interruptor. The observation consisted in finding the position of the slider for which no sound was heard in the telephone, or rather the two positions between which no sound was heard, for there was always a "dead" region. The tubes were immersed in the same water bath. The first observation was not taken for two hours after the tubes were put into the bath, so that the contents might be at the same temperature. So great is the temperature coefficient of electrolytes that a difference of half a degree between the tubes would cause at the lower temperatures an error of more than one per cent. It was therefore necessary to spend a great deal of time over each experiment. The temperature was now raised a few degrees above that of the room, and kept constant for a time, and then the bridge ratio was again determined. A few degrees of difference in the temperature caused very little change in the ratio. If the tubes were quite alike the ratio would always remain the same whatever changes the temperature might undergo provided that the two were always equally affected. As a matter of fact, the change in the ratio was very slight for a difference of 10° C. This was a great advantage. The bath was now emptied by a siphon and slipped from below the tube whose contents were not to be coagulated. After being filled again to the original level round the other tube it was heated till coagulation was complete. The water was again, siphoned off and the bath replaced so as to include both tubes, and refilled. It was thought worth while to go through this rather tedious process to secure the advantage of having both tubes in the same bath when the measurement was taken, and to avoid any movement of the tubes or their supports. An interval of several hours was allowed for the temperature to become the same in the tubes, and the bridge ratio was again determined for the temperature or temperatures at which it had been observed before. Any difference in the ratio must be due to change of resistance in the coagulated solution. The solution was not coagulated in the cups, so that there might be no chance of a change in the contact of the electrodes. The same routine could now be gone through again, the contents of the other tube being coagulated. Two experiments could thus be made with one filling of the tubes.

The following experiment is an example.

EXPERIMENT 1.—Egg-albumin.

	$\frac{R}{R'}$
Before coagulation	$\frac{2033}{1967}$
	$\frac{2032}{1968}$
After coagulation of tube A	$\frac{2033}{1967}$
	$\frac{2035}{1965}$
	$\frac{2035}{1965}$
	$\frac{2036}{1964}$
After coagulation of tube B	$\frac{2030}{1970}$
	$\frac{2033}{1967}$

The graduation of the wire was in millimetres. There were, therefore, 4,000 divisions in the whole length. An error of five divisions at the middle of the wire would only correspond to about a half per cent of error in the resistance. It was possible to say when the sound ceased in the telephone within a somewhat shorter range than five divisions, and the variations in the readings before and after coagulation are, as will be seen, well within this limit. The figures given are the middle points of the "dead space." We may conclude that if any change is produced by coagulation in a solution of egg-albumin which is strong enough to yield a firm coagulum, it does not amount to a half per cent of the total resistance. The coagulable material must therefore conduct very badly if it conducts at all; and the current must be carried mainly, if not entirely, by the non-coagulable constituents.

A solution of myosin in 10 per cent ammonium chloride gave a like result. The temperature was not allowed to rise beyond that required for coagulation. The conductivity of the solution was of course much greater than that of egg-albumin.

EXPERIMENT 2.—Myosin from dog's muscle, 10 per cent NH_4Cl.

Temperature 18° $\frac{R}{R'} = \frac{2004}{1996}$

After coagulation of A $\frac{R}{R'} = \frac{2004}{1996}$

EXPERIMENT 3.—Myosin from dog's muscle, 10 per cent NH_4Cl.

Temperature 18° $\frac{R}{R'} = \frac{2002}{1998}$

After coagulation of A

Temperature 8°·6 $\frac{R}{R'} = \frac{2014}{1986}$

18° $\frac{R}{R'} = \frac{2017}{1983}$

With the myosin solutions the readings did not generally agree with each other so well as in the case of egg-albumin. Whether this was due to the polarisation, which of course was far greater, being less completely eliminated, or to some slight decomposition taking place during the heating, I cannot say. The change of resistance on coagulation was sometimes so small as not to alter the ratio by a single division. Sometimes the alteration indicated a slight diminution, sometimes a slight increase of resistance. At most the change did not amount to one per cent.

Similar results were obtained by the second method. Here two tubes of the same cross section, but of different lengths were used. One was inserted in each coil of a differential galvanometer, a resistance box being included in the circuit of the shorter tube. The electrodes were platinum discs as before, but the ratio of their area to the cross section of the tubes was four times as great, for it was necessary to take advantage as much as possible of this means of diminishing the polarisation. The electrodes being of the same size, and the resistance being made equal in the two circuits, the polarisation would also be equal, and would therefore be eliminated. The resistance in the box corresponding to the null reading of the galvanometer would be equal to the difference of the resistances of the tubes. Both tubes were in the same waterbath, and when both were coagulated, the circuits would still be balanced, if coagulation causes no change in the resistance.

For the proper carrying out of the method, the two coils of the galvanometer must have the same resistance, and be so adjusted that the same current divided between them produces no deflection. If the coils are movable, as in the Wiedemann galvanometer, it is easy to adjust them. If they are fixed, probably the best mode of adjustment is to shunt the one which affects the mirror most, with a high resistance box till equality is obtained, always keeping the resistance of the system equal to that of the other coil by means of a separate box.

Experiments 4 and 5 are examples of a series of readings taken at various temperatures before and after coagulation. By plotting curves from such observations it is easy to see that there is no discontinuity at the temperature of coagulation. The resistance which has been diminished with increase of temperature rapidly at first, then more slowly, goes on diminishing above the temperature of coagulation, and there is no alteration in the curvature.

EXPERIMENT 4.—Egg-albumin. EXPERIMENT 5.—Egg-albumin.

Temperature.	Resistance.		Temperature.	Resistance.	
	Before Coagulation.	After Coagulation.		Before Coagulation.	After Coagulation.
11°	2100		11°	1880	1890
15°	1850		30°	1270	1270
20°	1670		40°	1070	1070
25°	1530		50°	935	
30°	1410		55°	860	870
35°	1310	1300	60°	810	
40°	1200	1200	65°	770	
45°	1120	1115			
50°	1030	1025			
55°	960	965			
60°	900	900			
65°	845	852			

The maximum change here is less than one per cent, which is still within the limits of error.

In the myosin solution as before the error is somewhat greater. The polarisation is not so completely eliminated as in the albumin solutions.

EXPERIMENT 6.—Myosin from dog's muscle, 10 per cent NH_4Cl.

Temperature 20°.	
Resistance before coagulation.	710
„ after coagulation.	710 720

EXPERIMENT 7.—Watery extract of dog's muscle.

Temperature 20°·1.	
Resistance before coagulation.	1420
„ after coagulation.	1415 1420

EXPERIMENT 8.—Blood Serum (dog).

Temperature.	Resistance.	
	Before coagulation.	After coagulation.
17°	3050	3075
25°	2790	2830
40°	2330	2240
50°	2100	2080

We see therefore that within the limits of errors of observation heat-coagulation of proteids produces no change in their resistance. Arrhenius has found that in a mixture of gelatine and salts no change in the rate of variation of the resistance with temperature is caused by the setting of the gelatine. The resistance of gelatine is therefore probably as great as that of coagulable proteids, and it is extremely likely that this is true also of the other albuminoids.

The direct method of investigation by comparison of the resistance before and after the abstraction of salts leads to the same conclusion as the indirect method by coagulation, and is of course applicable to any of the animal constituents.

The resistance of a standard tube of egg-albumin was determined. The albumin was then dialysed for a long time, concentrated on a water bath to its original volume, and the resistance again measured. It was found, as was to be expected, that the resistance increased with the length of time the dialysis was allowed to go on, increasing very rapidly in the first few hours, but always at a smaller and smaller rate as time went on. A dialysis of 48 hours has been found to increase the resistance eight to tenfold, and this although, as is now well known, it is impossible to obtain albumin entirely free from salts by dialysis. Experiment 9 gives a comparison of the resistance of a specimen of egg-albumin before and after dialysis, with that of a ·75 per cent solution of sodium chloride, all the measurements being made at the same temperature (12° C.).

EXPERIMENT 9.

Before dialysis.	809
After 30 hours' dialysis.	5952
·75 per cent NaCl.	933

EXPERIMENT 10.—A 2·6 per cent solution of commercial *peptone* had a resistance of 17 times that of a 2·6 per cent solution of sodium chloride. But it was not pure. It contained hemialbumose, but what is more important it was found to contain a trace of inorganic material, so that the resistance of pure peptone would be still greater. Peptone is therefore an exception to the general rule that easily diffusible substances conduct well, thus following its proteid affinities.

EXPERIMENT 11.—*Rabbit's muscle* was minced finely. One part was heated to 80° to coagulate the proteids, and the amount of ash in it was determined. The resistance of another portion which had been heated to 80° to diminish the polarisation as much as possible, was measured at 15°. It was then exhausted repeatedly with distilled water, and its resistance again determined at 15°. Finally a portion of the exhausted muscle was incinerated, and the ash weighed. The amount of ash was found to be originally about 1·1 per cent of the moist muscle, or 4·9 per cent of the total solids. The resistance of a standard tube of the muscle was 2600 ohms. After exhaustion with water the resistance was 12700 ohms, that is to say, the loss of the salt had increased the resistance fivefold. But this was not all. It was found that the exhausted muscle still contained ash to the amount of nearly 1 per cent of its total solids, or about ·2 per cent of the moist muscle. Keeping to whole numbers we see that a muscle with one-fifth of its salts has a resistance five times as great as if it had the whole of its salts. Now Kohlrausch has shown that in dilute solutions the resistance varies inversely as the percentage amount of the electrolyte. Muscle therefore behaves like a solution of its salts. Of course this statement is only approximately correct. It is only approximately correct even when polarisation is eliminated; and the living muscle has the capacity for taking on internal polarisation, which is impossible in a simple saline solution.

EXPERIMENT 12.—Rabbit's muscle was minced, and steeped in 20 times its bulk of distilled water for 72 hours, then washed with distilled water on a filter. Its resistance was compared with that of a ·5 per cent solution of sodium chloride, and the ratio between the two was found to be 60 to 17. This would correspond to the resistance of a solution of common salt containing about ·15 per cent. The amount of ash in a portion of the residue was found to be ·23 per cent of the moist muscle (·1 per cent soluble in water, ·13 per cent insoluble). The soluble ash was dissolved in water and the solution made up to the volume required to fill the standard tube which had been used in the experiment. Its resistance was found to be nearly equal to that of a ·07 per cent solution of common salt. We shall probably, therefore, be not far wrong if we assume that the specific resistance of the muscle minus its soluble constituents is less than that of a ·08 per cent solution of common salt. Since there is still inorganic matter in this residue, to the extent of ·13 per cent, the insoluble proteids cannot contribute much to the conductivity of muscle.

Ranke stated that boiling diminished the resistance of living muscle. Hermann, who afterwards discovered the enormous polarisability of muscle for transverse currents, conjectured that Ranke's result must have been due to the removal of this polarisability by the boiling, as he could find no marked difference of resistance for longitudinal currents between the living muscle and the muscle coagulated by heat. There seems no doubt that Hermann is right, and that Ranke's apparent change of resistance in heat rigor is due to a change in polarisability. I have not been able, any more than Hermann, to eliminate polarisation altogether for transverse currents, but the greater the speed of the interruptor the less was the difference between the cross resistance or the mixed cross and longitudinal resistance of the living and the heat-coagulated muscle.

Boiling undoubtedly does not diminish the true resistance.

On the contrary, it increases it by removing the salts. Rabbit's muscle, after being boiled in distilled water for 45 minutes, was found to have its resistance increased in the proportion of 13 to 22, and, after a repetition of the boiling with fresh water, the resistance rose to three times that of the original muscle, although the residue still contained nearly thirty per cent of the original ash.

Du Bois-Reymond showed long ago that in *rigor mortis* the resistance of muscle is apparently diminished. The observation certainly requires to be repeated with precautions for having the temperature throughout the muscle the same for the two resistance measurements, and especially with arrangements for eliminating polarisation. Otherwise since rigor diminishes the polarisability, the apparent resistance would be diminished even if the real resistance remained unaltered. The rise of temperature, so far as it goes, would act in the same direction. It is likely enough, however, that the true resistance may be changed in *rigor mortis* although it is unchanged by rapid heat rigor. For in the former the substances which cause the acid reaction are probably good conductors, and they are not likely developed at the expense of conductors equally good.

We see then that the conductivity of the salts is very great compared with that of the proteids of the tissues. But there are other substances of simpler composition than proteids, and many of them crystalline, the organic substances such as urea, uric acid, creatin, xanthin, lecithin, the fatty acids, and the carbohydrates, and we have to enquire what share they are likely to take in the electrolysis of an animal tissue or an animal liquid. At the outset it may be said that in general they are poor conductors, notwithstanding their comparative simplicity of formula, the rapid diffusibility of some of them, and their power of crystallisation.

A 2·6 per cent solution of grape sugar I found to have a resistance 142 times as great as that of a 2·6 per cent solution of sodium chloride. Cane sugar is a worse conductor even than grape sugar. Whether it be that there is a greater chance of slight saline impurities in the latter than in the former I do not know. But I found a 2·6 per cent solution of cane sugar to have no less a resistance than 905 times that of a similar solution of common salt. A starch solution has a resistance very much greater than that of cane sugar, and presumably glycogen is not a better conductor when it is pure. Of course the question arises here whether the starch or the glycogen can be got into true solution. I found a starch paste so bad a conductor that with an E. M. F. of 50 volts and electrodes of 50 square centimetres area at a distance of 3 centimetres, only a few bubbles were produced at the electrodes.

The carbohydrates may therefore be left out of account. The nitrogenous crystalloids like urea and uric acid, although better conductors than the carbohydrates, are far inferior to the inorganic salts. A 1 per cent solution of urea has, roughly speaking, a resistance twenty times as great as that of a 1 per cent solution of common salt; so that even in urine, which is so rich in urea, by far the greater part of the current will be carried by the salts.

Animal fats are exceedingly bad conductors. A piece of beef fat had such an enormous resistance that I had not the means of measuring it, and this, although the electrodes and the ends of the prism of fat were moistened with salt solution. Adipose tissue in the living animal is in quite a different position. It conducts by means of its blood vessels, its connective tissue and its fluids, which are continuous with those of the strata above and below it.

We may take it as proved that in artificial mixtures of proteids, inorganic salts, and nitrogenous extractives, in solution in such proportions as they are found in the fluids of the animal body, by far the greatest part of the current passes by decomposition of the inorganic substances. In other words, so far as the *direct* effect of the current is concerned, electrolysis of such solutions is practically equivalent to electrolysis of the salts contained in them. The *direct* decomposition of the other constituents is at any rate so small, that, if not insignificant in itself, it is of little consequence when compared with the secondary and *indirect* effects of the products of electrolysis of the salts.

For physiological liquids, such as blood-plasma, lymph, cerebro-spinal fluid, the digestive juices, bile and urine; for pathological transudations such as ascitic fluid, hydrocele fluid, and pericardial fluid; for pus or at any rate for the plasma of pus, we need have no hesitation in extending the results gained with similar liquids outside the body to the case where these fluids form part of a heterogeneous circuit inside the body, and *a fortiori* to the case where they are directly tapped by the electrodes.

But the electrolysis of a physically heterogeneous tissue is a more complex subject than the electrolysis of a physically homogeneous liquid. Take a muscle, for example. No doubt its cells are bathed with blood and lymph, and these liquids inside the muscle, so far as they are continuous, will conduct as they would outside the body. But when we take the muscle as a whole we have no longer the simplicity of physical homogeneity. The phenomena of internal polarisation appear; the ions are shed out upon a multitude of secondary electrodes within the substance of the tissue; and this polarisation, combined as it may be with secondary transition resistances, may cause a distribution of the current which does not correspond to the resistances of the constituents taken separately. But whatever the real significance of this internal polarisation may be (its amount seems too great for a polarisation at the boundaries of dissimilar electrolytes), its existence is a confirmation of the view arrived at from other evidence, that by far the greatest part of the conductivity in the tissues is electrolytic. For it points to abrupt changes of potential within the muscle, and therefore indirectly to abrupt changes of conductivity.

The proteid constituents of the tissues then are only to a small extent, if at all, affected by the direct electrolytic action of the current. But we know that the most profound changes, immediate or ultimate, may be wrought in a normal tissue such as frog's muscle, or in a vascular tumour like a naevus or a goitre by a single application of a not very powerful current. (See an interesting address by John Duncan, *Brit. Med. Jour.* Nov. 3rd, 1888). How are these changes brought about? Considering only the physico-chemical action of the current, we may say that there are three possible ways in which it can produce its effects; (1) by the action of the products of electrolysis given off at the electrodes, or in the mass of the tissue if there is internal polarisation; (2) by the removal of salts from the tissue by decomposition; (3) by cataphoric action; and (4) by raising the temperature of the part. We shall consider these possible modes of action in order, citing the experimental evidence which bears upon each.

(1) *The action of the products of electrolysis of the salts.*

It has of course long been known that when an animal solid or liquid is electrolysed substances of acid reaction appear at the anode and substances of alkaline reaction at the cathode. The greatest part of the alkali probably consists of sodium and potassium hydrate. Bubbles of hydrogen gas are given off owing to the decomposition of the water by the cathions. At the anode acids are formed by the action of the anions on water and gas is given off in most cases. It consists chiefly of oxygen, but chlorine is always present as well.

In order to ascertain the kind of action which these electrolytic products may have, I made a series of experiments, partly on liquids (blood and bile) containing a pigment the changes in which could be readily observed, partly on proteid solutions and on isolated tissues.

Electrolysis of blood. More than a quarter of a century ago Rollett observed that discharges from a Leyden jar caused defibrinated blood to become transparent, the hæmoglobin coming out of the corpuscles and going into solution. (*Wien. Sitzungsber.*, XLVI., 1862). He stated that the constant current and induction shocks of small electromotive force did not have this effect. (*Ibid.*, 1863.) Neumann found, however, that ultimately induction shocks caused the same change as the Leyden jar discharge. He explained it as due to the action of ozone produced by the discharge. Rollett (*Ibid.*, 1864, 1865) and Schmidt (Virchow's *Archiv*, 1864, p. 1) investigated the action of the voltaic current, the former giving a minute description of the microscopic changes. Schmidt again used the theory of the production of ozone to explain the effects. Rollett in a later publication (Hermann's *Handbuch der Physiologie*) puts forward the view that hæmoglobin acts the part of a weak acid in electrolysis, and is actually carried as anion to the positive electrode. Schmidt found that when dog's or horse's blood was electrolysed, a layer of material in which masses of hæmoglobin crystals were embedded collected on the positive electrode. In dog's blood he found that the formation of the crystals began in less than a minute after the current was closed. (Apparently he did not measure the strength of the current, but it could not have been a very strong one from his description.) Rollett found the same phenomenon, and on the strength of this and of Preyer's discovery that hæmoglobin has a slightly acid reaction (Pflüger's *Archiv*, I. p. 405), he concluded that the hæmoglobin is carried to the anode without decomposition. Whether this is the case we shall see later on, but the mere fact of the appearance of hæmoglobin crystals near the anode does not prove anything more than that the conditions there are favourable to crystallization of the hæmoglobin. We shall be in a better position to discuss the cause of the striking changes produced after some of the experiments have been described, and to these we now proceed.

EXPERIMENT 13.—A current of 45 milliampères, 6½ milliampères per square centimetre of section, was passed through defibrinated rabbit's blood for 3 hours. The electrodes were platinum discs, each having an area of 7 square centimetres. That the action of the two poles might be separated, one of the electrodes (the anode in this experiment) was contained in a porous clay pot. This was placed in the vessel containing the other electrode, and both vessels were filled with blood to the same level.

Very soon after the closing of the current the reaction of the blood around the anode, which was alkaline to begin with, changed to acid. In an hour the acidity was very marked. Around the cathode there was a corresponding increase in the alkalinity. There was a brisk evolution of gas at the cathode. At the anode much less gas was given off. Schmidt saw no gas given off from the anode. This is probably because he used weaker currents. I have always found that some gas is given off at the anode, and the relative amount increases when the blood is diluted with distilled water. At the end of the experiment the whole of the blood in the vessel which contains the anode forms a tarry-looking mass, which is seen under the microscope to consist largely of red blood corpuscles, altered in shape and some of them deprived of hæmoglobin, mixed with amorphous yellowish pigment. The mass is soluble in distilled water only to a very slight extent. After being well washed with water it is neutral or slightly acid to litmus paper. A layer of it pressed between two glass slides without the addition of any solvent shews a spectrum with a single well-marked band in the red in the position of the acid-hæmatin band. When the mass is rubbed up with water and filtered, the filtrate has a slight yellowish brown tinge. The residue is partially soluble in dilute caustic soda and also in dilute acetic acid, and the solution in the former gives the spectrum of alkali-hæmatin. A solution which is not strong enough to shew this spectrum gives nevertheless a splendid spectrum of hæmochromogen on the addition of a drop or two of ammonium sulphide. The acetic acid solution gives the spectrum of acid-hæmatin.

At the negative electrode there is no mass deposited. The blood becomes laky, the hæmoglobin going into solution. It may be turbid from the presence of a brown amorphous material, which is filtered off with difficulty. The filtrate, when the electrolysis has not gone very far, shows the spectrum of oxyhæmoglobin. After the current has been passed for some time the band of alkali-hæmatin appears at *D*, the oxyhæmoglobin bands still persisting. The addition of a drop or two of ammonium sulphide very rapidly changes the spectrum to that of hæmochromogen. If the liquid, instead of being filtered, is cleared up by the addition of a drop of ammonia, the same spectrum is seen as in the filtered liquid. On neutralising with dilute acetic acid there is a copious precipitate, which is soluble in excess of the acid, giving the spectrum of acid hæmatin. When the alkaline liquid is boiled there is some coagulation. This is increased by acidifying with dilute acetic acid.

EXPERIMENT 14.— When blood diluted with its own volume of distilled water was electrolysed by a current of 250 milliampères for 30 minutes in an apparatus in which the electrodes were separated by a partition of animal membrane, a thick layer of hæmatin was deposited on the surface of the membrane next the anode. The reaction of this was neutral to litmus paper. The liquid in the anodic compartment was acid, and gave the spectrum of acid-hæmatin. The liquid in the cathodic compartment was turbid from the presence of flakes of hæmatin. When filtered, it showed a spectrum with the two oxy-hæmoglobin bands, and the band of alkali-hæmatin over *D*. There was a thin layer of almost colourless material on the anode, which behaved like a coagulated proteid. The liquid in the anodic compartment contained no coagulable proteid, while that in the cathodic compartment gave a considerable coagulum on heating, which was increased on acidulation with dilute acetic acid. Methæmoglobin appears at the anode before acid-hæmatin.[1]

We see from these experiments that at the positive pole the hæmoglobin is acted upon, one of the products being acid hæmatin. In all probability the globulin constituent is deposited on the anode in the coagulated form. Part of it may be changed into acid albumin. The proteids of the plasma undergo similar changes. At the cathode the action is not so marked. Part of the oxyhæmoglobin is certainly changed into alkali-hæmatin, but some of it, in many experiments, remains unaltered. There is no coagulation of proteid substances. Part of the proteids passes into alkali-albumin, part remains unchanged.

We must now consider how these changes are brought about.

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[1] When the electrolysis is done slowly with a weak current in a large U-tube or in a couple of parallel-sided glass troughs connected by an inverted U-tube filled with diluted blood, a stage may be found when at the anode methæmoglobin and oxy-hæmoglobin are apparently alone present, and ammonium sulphide gives the spectrum of reduced hæmoglobin. Later on the oxyhæmoglobin bands disappear, and methæmoglobin and acid-hæmatin seem to be present together. Ammonium sulphide then gives the band of reduced hæmoglobin along with the hæmochromogen bands. The oxyhæmoglobin bands may spring out for an instant on the addition of the ammonium sulphide.

In particular, how is the hæmoglobin affected? By direct electrolysis or by the products of the electrolysis of other bodies?

In answering these questions the first step was to get some idea of the conductivity of a pure hæmoglobin solution.

It was besides of considerable theoretical interest to determine the resistance of a crystalline colloid, as it is so difficult to separate ordinary colloids from crystalloid impurities. It was scarcely to be expected that hæmoglobin should be a good conductor.

It is a substance which diffuses badly, its composition is more complex even than that of the proteids, which, as we have seen, are very bad conductors, and the power of crystallisation cannot prevent cane sugar and many other organic substances from acting almost as insulators. The crystals were obtained from the blood of the goose, were crystallised and recrystallised several times and well washed. The solution was concentrated before crystallisation by freezing. A mass of pure ice was formed which could be lifted out, leaving nearly all the hæmoglobin in strong solution behind. This proved a good plan, and seems specially adapted for working with substances like hæmoglobin, which must be manipulated at a low temperature. A 4 per cent solution was found to have a resistance 16 times as great as that of a ·75 per cent solution of common salt. Probably the real resistance of the hæmoglobin was still greater. For after the separation of the hæmoglobin by heat-coagulation and filtration, the resistance of the filtrate, made up to the original volume of the solution, was increased only in the proportion of 39 to 27.

On the assumption that in the coagulation of the hæmoglobin no decomposition occurred which increased or diminished the resistance of the filtrate, the true resistance of the hæmoglobin must have been $\frac{39}{12} \times 16$, *i.e.*, 52 times that of a ·75 per cent solution of sodium chloride.

It is doubtful, however, whether the assumption is legitimate, for we know that small quantities of formic and butyric acids and other substances are formed when hæmoglobin is decomposed in this way. Experiments with other specimens of hæmoglobin have been made where the resistance of a 4 per cent solution was found to be 15 times that of a ·75 per cent solution of common salt, and 75 times that of a 4 per cent solution. In any case the resistance is very high, so high that the distilled water used for making the solution, unless specially prepared with elaborate precautions against impurities, may itself conduct a sensible proportion of the current. This renders it very improbable that hæmoglobin is carried undecomposed to the positive pole as Rollett suggested, and that the crystals which Schmidt saw gathering in the coagulum on the anode were due to such a heaping up of the hæmoglobin. Experiments to be afterwards described shew that there is no electrolytic transfer of this kind in hæmoglobin solutions, but that the changes produced in the pigment are due to secondary actions.

If we take the conductivity of blood as about equal to that of a ·75 per cent solution of sodium chloride, that of the hæmoglobin in the blood, (9 per cent say), if it were in the same state as regards conductivity as in pure aqueous solution, would not probably be at most more than a tenth of the whole. Now taking the electro-chemical equivalent of chlorine as ·00353, with a current of 100 milliampères the amount of chlorine evolved at the positive pole in the electrolysis of a chloride would be ·0000353 gramme per second, or ·00211 gramme per minute. Let us assume now that in the blood the current is divided according to the conductivity of the various constituents, an assumption which would not lead us far astray if the blood were physically homogeneous, *i.e.* if the constituents of the corpuscles, instead of being concentrated in circumscribed systems suspended in a liquid of uniform composition, were distributed uniformly through the plasma. With a total current of 100 milliampères not more than 10 milliampères would be carried by the hæmoglobin, a current which would suffice to separate only ·000211 gramme of chlorine per minute from a chloride. Now there are about 9 grammes of hæmoglobin in 100 c.c. of blood. If the electro-chemical equivalent of hæmoglobin was no greater than that of chlorine, a current of 100 milliampères would require something like 30 days to directly electrolyse the hæmoglobin of 100 c.c. of blood. But a two to four hours' electrolysis of 100 c.c. of blood with a current of this strenth is sufficient not only to bring all the hæmoglobin out of the corpuscles but also to effect further profound changes. It may be said that the electro-chemical equivalent of hæmoglobin, if it can really act as an ion, is probably very much greater than that of chlorine, and that the direct action of even a feeble current is adequate to the transference of large quantities of it to the positive pole. The following experiments shew, however, that there is no such transference, and that when arrangements are made to prevent the secondary actions at the electrodes, the hæmoglobin is not affected at all by the current.

EXPERIMENT 15.—A glass U-tube was partly filled with a 3·4 per cent solution of oxyhæmoglobin. Ordinary tap water was carefully introduced above this in each limb of the tube, and remained unmixed with the hæmoglobin. The platinum electrodes dipped into the water, but did not extend so far down as the top of the hæmoglobin solution. A current from a battery of 7 Leclanché cells was passed for an hour and a half. Its strength was only a fraction of a milliampère. No change whatever took place in the distribution of the hæmoglobin. At neither electrode did the water become tinged, nor did the spectrum of the solution show any alteration.

That this was not due to the feebleness of the current is shewn by the following experiment.

EXPERIMENT 16.—The same U-tube was filled with the hæmoglobin solution, the electrodes dipping directly into it. A current from the same battery was passed through it. The current was again only a fraction of a milliampère, too small to be accurately read. It could not differ much in strength from that used in the previous experiment, for the resistance of the tap water was not very different from that of the hæmoglobin solution. Nevertheless, in 70 minutes the liquid in the upper third of the anodic limb around the electrode was almost completely decolourised, and a layer immediately below this was distinctly more dilute than the original solution, had a brownish tinge, and shewed a spectrum with a well-marked band in the red, besides the two oxyhæmoglobin bands. Flakes of brown material, which was afterwards found to be hæmatin, had fallen down from the liquid in the anodic limb, and remained in the bend of the tube. In the cathodic limb no change could be observed either in the spectrum or in the strength of the solution. Bubbles of gas were still being given off at both electrodes, especially at the cathode. The reaction, which before the electrolysis was faintly alkaline, was now decidedly acid at the anode and alkaline at the cathode.

In two hours more, the whole of the anodic limb was decolourised, and the bend of the tube was filled with whitish brown flakes. There was no change in the cathodic limb. The changes at the anode must be due to the action of the electrolytic products; and the relatively small quantity of gas given off shews that either the primary products are prevented from decomposing the water by the presence of some other substance which is attacked by preference, or that the nascent oxygen is itself seized upon by such a substance. This substance can be no other than the hæmoglobin. Experiments 17 and 18 complete the proof, for they shew that even when one electrode dips directly into the hæmoglobin solution, the other being in clear water, there is no trace of any transference.

Experiment 17.—The U-tube was filled with the hæmoglobin solution, except the upper part of the cathodic limb which had tap water in it. The cathode did not reach the solution. Everything proceeded just as before in the anodic limb. The water around the cathode did not take up the slightest tinge of colour. Accordingly, none of the pigment is transferred to the cathode.

Experiment 18.—The U-tube was filled with the solution, except the upper part of the anodic limb, which contained water. No change in the pigment took place in any part of the tube. Accordingly, none of the pigment is transferred to the anode.

We conclude, therefore, that *the hæmoglobin is only affected by the products of electrolysis which appear at the anode, and is not directly affected by the current.* The secondary action at the anode is always much greater than at the cathode, and with weak currents there may be no recognisable change at the latter except the increase of alkalinity. For this reason, the effect of the cathode was much less marked with the hæmoglobin solutions than with blood; a given electromotive force producing of course a much stronger current through blood containing all its salts than through the hæmoglobin solution with only accidental traces of them. When sodium chloride was added to the hæmoglobin solution till its conductivity was equal to that of blood, there was no observable difference in the nature and amount of the spectral changes, but the anode was always more powerful than the cathode.

How are we now to figure to ourselves the mechanism of these extraordinary polar effects? Does the electrolysis simply place at the electrodes free acids and alkalies, which then act upon the hæmoglobin as acids and alkalies of the same strength and kind would, if introduced, from without? This is a question which Schmidt asked with reference to the solution of the corpuscles and the liberation of the hæmoglobin from them in the electrolysis of blood. He thought it incredible that the comparatively small amount of alkali set free at the negative electrode could effect such a change, and explained the solution both there and at the anode as due to the action of ozone. What share ozone may or can take in the process I know not. It is credible that ozone, produced in the electrolysis of blood, after causing the hæmoglobin to pass out of the corpuscles, should break it up, and that the other electrolytic products should change it into acid-hæmatin at the anode, into alkali-hæmatin at the cathode. But it is scarcely credible that ozone produced in the electrolysis of egg-albumin or of myosin should coagulate some of it upon the anode, change some of it there into acid-albumin, and cause the formation of a great quantity of alkali-albumin at the cathode, and that all through those electrolytic operations on tissues and individual constituents of tissues, two opposite kinds of effects should be produced by a single chemical agent. Yet when the process is watched simultaneously in blood and in artificial proteid solutions and the two kinds of changes are seen to advance in each with equal step,—at the cathode gradually increasing alkalinity, with solution of the corpuscles and formation of alkali-hæmatin in blood, gradually increasing alkalinity with the formation of alkali-albumin in simple albuminous solutions; at the anode, growing acidity with coagulation of the corpuscles, and formation of acid-hæmatin, growing acidity with coagulation of albumin and formation of acid-albumin—it is difficult to resist the conclusion that in both cases there is a causal connection between the changes in reaction and the other effects. If the alkalies arising from the decomposition of the slender stock of salts in egg-albumin are adequate to the task of converting the albumin into alkali-albumin, what is to prevent the alkalies formed in the electrolysis of blood from dissolving the red corpuscles?

But even if it be granted that the changes in blood are brought about by the ordinary products of the electrolysis of the salts, it must still be considered whether these products are primary or secondary, whether they act in the nascent or in the free state.

Experiment 19.—A 3·6 per cent solution of hæmoglobin was electrolysed in a U-tube. As soon as the anodic limb was decolourised the clear acid liquid was removed by means of a pipette. A few drops of hæmoglobin solution were added to it; and the spectrum observed. It shewed only the oxy-hæmoglobin bands of the original solution. In a quarter of an hour there was still no acid-hæmatin band. Gradually, however, in the course of several hours this band appeared, the oxy-hæmoglobin bands disappeared, and the addition of a drop or two of ammonium sulphide immediately produced the bands of hæmochromogen. Here, then, we had a solution containing all the electrolytic products at the anode, except what had gone off as gas, or might have been used up in effecting changes in the hæmoglobin. This solution only very gradually affected unaltered hæmoglobin, far less rapidly than it is affected when the current is passing. Either then it does not contain the most active products, or it does not contain them in the most active form.

It is certain that active oxidation goes on at the positive electrode. The absence of gas or its small relative amount indicates that the nascent oxygen is intercepted by some easily oxidisable material. Chlorine, which is a constant product of the electrolysis of animal tissues, comes off sparingly or not at all when blood is electrolysed. But chlorine must be carried as anion, since blood contains chlorides. What becomes of it? We can hardly doubt that it acts upon the blood pigment, that it oxidises it by decomposing water in its presence or possibly by attacking directly its molecular hydrogen. The nascent oxygen corresponding to sulphion and the other anions which re-form their acids at the anode, must be intercepted by something which it oxidises; and what can that something be in a hæmoglobin solution with saline impurities except the hæmoglobin itself? Weak chlorine water produces at once the changes in hæmoglobin which are caused gradually at the anode by the passage of a current. The colour changes to yellowish brown, the oxyhæmoglobin bands disappear, and the spectrum may shew no bands at all, or only a band in the red to the right of C. The addition of a few drops of ammonium sulphide will, however, bring out a well marked hæmochromogen spectrum. Strong chlorine water will decolourise the solution altogether, just as a strong current will do. The following experiment is interesting in this connection.

EXPERIMENT 20.—A solution of oxyhæmoglobin was cautiously reduced by ammonium sulphide, a slight excess of the reagent being added. The solution was now electrolysed in the U-tube by a current of half a milliampère. No gas whatever was given off at the anode, although with an oxyhæmoglobin solution of the same strength a weaker current always caused a slight evolution of gas there. In two hours there was no change in the anodic limb, but at the cathode great part of the liquid had become of a pale yellow colour.

This is the reverse of what happens with a solution of oxyhæmoglobin. We cannot suppose that it is the electrolysis of the ammonium sulphide which causes the difference, for the addition of ammonium chloride does not affect the normal process in an oxyhæmoglobin solution, except by hastening it.

The oxidising agent, nascent oxygen, let us say—Schmidt would say ozone, and however small the amount of ozone may be it will undoubtedly play a part—does not affect the hæmoglobin round the anode because it is taken up by the ammonium sulphide. The liberated acids do not attack the hæmoglobin because the reaction is kept alkaline. At the cathode the absence of the loosely combined oxygen of the oxyhæmoglobin appears to condition the ultimate destruction of the blood pigment. We may suppose, therefore, that oxyhæmoglobin is saved from the attack of reducing agents given off at the cathode by parting with some of its loosely-combined oxygen, and thus preserving its molecular oxygen intact.

EXPERIMENT 21.—A trace of ammonium chloride was added to an oxyhæmoglobin solution. The conductivity was of course greatly increased. A current of two milliampères caused the solution around the anode to become pale yellow in ten minutes. Gas came freely off from both electrodes, and a faint smell of chlorine could be detected at the anode. The solution at the cathode was not at all affected. The only effect of the addition of ammonium chloride was greatly to hasten the process which goes on in the simple oxyhæmoglobin solution. Gas appears at the anode apparently because the increased conductivity causes a much stronger current for the same E.M.F., and there is not hæmoglobin enough to be acted upon by all the primary or secondary electrolytic products, some of which must come off as gas. When the current is reduced to the strength which it had before the addition of ammonium chloride, everything goes on practically in the same way as in the simple hæmoglobin solution. Therefore ammonium sulphide in the previous experiment does not alter the action because it is an ammonium salt. It is not the ammonia given off at the cathode which produces the change there, for with the same current as much will be given off from the ammonium sulphide solution as from the ammonium chloride one. But the presence of free ammonia in the solution has a notable effect.

EXPERIMENT 22.—A trace of dilute ammonia was added to a solution of oxyhæmoglobin. The spectrum remained that of oxyhæmoglobin. Electrolysis for two hours with a very weak current produced changes similar to those produced in the ammonium sulphide solution. The cathodic limb of the tube was almost decolourised, the anodic remaining unchanged. But gas was given off freely at both electrodes. The presence of free alkali seems therefore to protect the hæmoglobin from the action of the products given off at the anode, presumably by uniting with the anions. The reaction was still alkaline at the end of the experiment in the whole tube.[1]

EXPERIMENT 23.—An oxyhæmoglobin solution was made acid with acetic acid. It was electrolysed for an hour by a current of 2 milliampères. Gas was given off at both electrodes. The anodic limb shewed a distinct change in the strength of the solution, while there was no change elsewhere. We cannot conclude from this that the change in the oxyhæmoglobin at the anode is favoured by an acid, and checked by an alkaline reaction. For the spectrum shewed that acid-hæmatin was present; and that more than the reaction is concerned is seen from the following experiment.

EXPERIMENT 24.—A solution of hæmoglobin reduced by carbonic acid gas was electrolysed by a current of half a milliampère. The reaction was decidedly acid and the spectrum shewed a well-marked acid-hæmatin band in the red, along with the band of reduced hæmoglobin. A great deal of gas was given off at the anode, apparently as much as at the cathode. At the latter it did not go off freely, but collected in bubbles on the electrode or in a precipitate which formed on it. In an hour the cathodic limb was decolourised to the level of the lower edge of the electrode, which was covered by a brown deposit. The reaction was faintly acid at the cathode, approaching neutrality. The current was now reversed, and now the solution in the former anodic limb was affected in exactly the same way as the cathodic limb had been affected before—and that although the reaction remained acid till the end.

The proteids of the serum and the stroma of the corpuscles undergo changes which are difficult to follow in entire blood.

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[1] When a trace of ammonia is added to diluted blood in the cathodic limb only of the U-tube the blood in the anodic limb is no longer protected. If ammonia is added only to the anodic limb it is protected.

At the anode undoubtedly part of the proteids of the serum is coagulated and helps, with masses of altered corpuscles, to form that layer on the electrode which is so conspicuous a result of the process; but another part passes into the state of acid-albumin. The stroma of the corpuscles goes into solution at the cathode, forming presumably alkali-albumin. As to the globulin element of the hæmoglobin, some of it is certainly precipitated about the anode in a coagulated form. Whether any of it is changed into acid-albumin I cannot say. It is to be expected that some of it may undergo this change; for in dilute solutions of hæmoglobin the alterations in the pigment may be completed without any precipitation at the anode.

Experiment 25.—The serum of horse's blood, obtained by allowing the corpuscles to subside after the addition of leech extract, was electrolysed by a current of 20 milliampères mean strength, or 3 milliampères per square centimetre of section. Much gas was given off at the anode as well as at the cathode. Serum differs in this respect from blood, as was pointed out by Schmidt. Undoubtedly the absence of hæmoglobin from the serum is the cause of the difference; and it shows how readily hæmoglobin is acted upon by the anions themselves or by the oxygen produced by their action upon water. We have already shewn that under certain conditions hæmoglobin or its derivatives may be present, and yet be shielded from the action of the electrolytic products. After an electrolysis of only 30 minutes a large proportion of the proteids of the serum in the anodic compartment was found in the form of acid-albumin, the reaction being acid. There was a small coagulum on the anode, filled with bubbles of gas and smelling of chlorine. It was completely soluble in dilute caustic soda in the cold, much gas coming off as solution went on. It was not at all soluble in acetic acid. Some coagulable material still remained in solution. In the negative compartment there was a considerable amount of neutralisation precipitate, but still more of the proteids remained in a coagulable form. As usual the reaction was strongly alkaline, and there was no deposit on the cathode.

The proteids of the serum, as we shall see later on, are affected in the same way as the proteids in the simpler solutions, such as egg-albumin, or myosin dissolved in sodium chloride. It may be, however, that in entire blood the presence of hæmoglobin, which is so readily attacked by the products of electrolysis, may to some extent shield the proteids, and lessen the amount of change produced in them by a given current, but the kind of change it does not seem to affect.

The electrolysis of blood naturally stands first in a systematic investigation of the electrolysis of animal tissues, for blood and the parenchymatous juices form part of every circuit which includes an animal body. Of the solid tissues, muscle, on account of its wide distribution and the immense share of the current which must pass by it in every deep electrolysis, claims the first place. But before we take it up it will be well to place here immediately after blood an account of experiments on two proteid-free animal liquids, bile and urine. Taken by themselves, these are of no importance whatever, but they supply evidence of the *modus operandi* of electrolysis which is of interest in connection with the changes which have been described in blood, and those which are about to be described in muscle.

Electrolysis of Bile.

The effect on the pigments is very striking, and reminds one of the electrolysis of blood. The oxidising action of the anode is well marked, bilirubin being oxidised first to biliverdin, and beyond this, with a strong current, to colourless or almost colourless products. The biliverdin can be again reduced to bilirubin by reversing the current, and thus exposing it to the action of the cathode. When this action is allowed to go farther the bilirubin may be changed into a yellow pigment.

The mucin is precipitated on the anode by the liberated acids. Around the cathode it is partly changed into alkali-albumin. Again we see that the action is a secondary action. It is just possible that the bilirubin may to a small extent conduct the current, for it forms combinations with bases, and is of comparatively simple composition. Its chloroform solution is not a good conductor. The bile salts conduct better than any of the other nitrogenous metabolites which I have examined. A 2·3 per cent solution of mixed bile salts had a resistance less than twice that of a ·5 per cent solution of common salt. It contained ·25 per cent of ash. A volatile alkali in minute traces was given off at the cathode when bile was electrolysed. Possibly this came from decomposition of the bile acids. The comparatively high conductivity of bile points to the same conclusion. (For a more detailed account see paper on "The effect of electrolysis and of putrefaction on the bile, and particularly on the bile pigments," p. 178).

Electrolysis of Urine.

EXPERIMENT 26.—A current of 10 milliampères per square centimetre of section was passed through fresh urine for 50 minutes. The reaction which was acid at first, became in 7 minutes faintly alkaline in the negative compartment and strongly acid in the positive. At the end of the experiment the colour of the urine at the anode was brownish. It looked and smelt just like urine to which hydrochloric acid had been added. There was an amorphous sediment coloured with the pigment, and consisting partly of urates. There were no uric acid crystals. The sediment was entirely soluble in dilute alkalies. At the cathode the colour of the urine was unchanged. There was a deposit of beautiful crystals of stellar phosphate mixed with amorphous material, the whole being soluble in dilute acetic acid.

EXPERIMENT 27.—For the more exact determination of the nature of the precipitates fresh urine was evaporated on the water-bath to one-fifth of its volume, and filtered. A current of 40 milliampères per square centimetre was passed through the filtrate for 85 minutes. The reaction, strongly acid at first, was found to be alkaline in the negative compartment in 50 minutes after the closure of the current. Much gas was given off at both electrodes. A piece of litmus paper held over the cathode was turned blue, and the smell of ammonia was distinctly perceived, while at the anode chlorine was recognised. At the negative pole there was a profuse deposit of triple phosphate in the dendritic form, along with some small coffin-lid crystals and crystals of stellar phosphate. The whole deposit was soluble in dilute acetic acid. At the positive electrode, the change in colour and smell described in the last experiment was even better marked. The sediment consisted chiefly of brown amorphous masses, with a few brownish balls of sodium urate aggregated in groups of two or three, or scattered singly through the field. The current was now reversed. The reaction of the former cathode gradually became less alkaline and finally acid, and with the change of reaction the deposit of phosphates disappeared, and brown balls of sodium urate took their place. Corresponding' changes took place at the former anode, the urate disappearing, and amorphous phosphates being thrown down. Even while the reaction is still acid, volatile alkali may be given off. In this case the layer next the electrode is probably neutral or alkaline, which is a hint that at the very surface of the electrodes, the secondary actions may have an intensity quite out of proportion to the total amount of chemical change, considered as uniformly distributed through the whole conductor. And, even in what is practically in this connection a mixture of inorganic salts, the direct decomposition may be trifling compared with the indirect effects.

We come now to muscle.

Electrolysis of Muscle.

The polar effects are here more sharply localised than is the case in solutions, for, apart from electric cataphoresis, interchange can go on only by diffusion, and not by convection currents. There are four ways in which electrolysis can affect in the living body such a tissue as muscle by its chemico-physical action: (1) by the chemical effects of the products liberated at the electrodes or in the substance of the tissues; (2) by the removal of electrolytes (chiefly inorganic substances) which are necessary for the vitality of the tissue, leading to increased absorption of these elements, and perhaps, in consequence, to increased activity of general nutrition in the tissue, or, if the action be more intense, to diminished vitality, degeneration, or death; (3) by cataphoresis, leading to increase of the transferred substances around the cathode, diminution around the anode, with the consequent increase of eliminating or absorbing activity by which a tissue returns to healthy equilibrium, or to any of the results which follow a disturbance of that equilibrium beyond the limits of health; (4) by raising the local temperature.

(1) *The Chemical Effects of the Liberated Products of Electrolysis.*

EXPERIMENTS 28—30.—A current initially of 35 milliampères, 5 milliampères per square centimetre of section, was passed for two hours through a glass trough filled with living frog's muscles. The distance between the platinum electrodes was 10 millimetres. The muscles were cut into small pieces so that the trough could be easily filled with them. A sample was kept for control. For 17 minutes the muscle still remained excitable. There was a strong smell of chlorine at the anode. At the end of the experiment the current had sunk to 5 milliampères although the electromotive force was the same as at first, The muscle consisted of two layers of nearly equal thickness marked off from each other by a fairly definite line of junction parallel to the electrodes. The cathodic layer was strongly alkaline throughout its whole depth, and had a brownish tinge. The anodic layer was acid. It was almost colourless.

Microscopic Appearances.

In the anodic layer the most striking change was the great distinctness of the nuclei. Great numbers of them were seen. It was just as if acetic acid had been allowed to act on the muscle. The fibres were everywhere bounded by a sharply defined and uninjured sarcolemma, no matter how great the change in the contents might be. The sarcolemma, which resists the action of chemical agents so much, resists also the action of electrolysis, a fresh indication that it is the secondary chemical changes which are chiefly concerned, at least in the immediate effects of the current. The contents of the fibres shewed every gradation of change. In some there was complete destruction, the striation and even the nuclei being gone, and nothing remaining within the sarcolemma except a granular structureless mass. But this was comparatively rare. Many of the fibres shewed no change in the transverse striation, and in fact no change at all except for the prominence of the nuclei. In many the transverse situation could still be made out, but less clearly than in the normal muscle. The longitudinal striation, on the other hand, was well marked, and could sometimes be seen where the transverse was entirely destroyed. These changes were not confined to the surface next the electrode, but penetrated through the whole thickness of the anodic layer.

In the cathodic layer there was first a thin stratum next the electrode, consisting of clear jelly-like material. The fibres in this layer shewed, for the most part, neither nuclei nor transverse striation, but the longitudinal striation was quite distinct. The sarcolemma was well marked, and the contents were not granular, but nearly homogeneous. A few nuclei were seen, but very few. This may be called the region of utter destruction. In the rest of the cathodic layer the state of the fibres resembled that of the anodic layer, except that the nuclei were not seen. The transverse striation was sometimes unaltered, often greatly impaired or destroyed. A few fibres shewed only homogeneous contents. It is to be noted that destruction of the fibres in the anodic layer rendered the contents granular, as if it were a coagulation, while destruction in the cathodic layer was associated with a homogeneous appearance of the fibres, as if it were due to a solution of the contents or a chemical alteration other than coagulation. Chemical examination confirmed this impression.

EXPERIMENT 31.—Muscle electrolysed as in the last experiment was separated into its two layers. After being rubbed up in a mortar, the portions were extracted for 17 hours with water. After the watery extract had been filtered off the residue was washed on the filter with water, and afterwards extracted with 10 per cent sodium chloride solution for 24 hours. The muscle which had not been electrolysed was treated in the same way. The percentage amount, of the various proteid constituents, reckoned on the weight of the moist muscle, was as follows:—

	Watery Extract.		NaCl Extract.
	Neutralisation	Coagulable material.	Coagulable material.
Anodic layer.	—*	·50	·31
Unelectrolysed.	—*	1·12	1·80
Cathodic layer.	·50	·36	·29

* Too small to reckon.

From experiment 31 we see that the neutralisation precipitate, that is to say the alkali-albumin, is much increased in the cathodic region, while the coagulable proteids soluble in water are diminished. There is no reason why they should be *proportionally* diminished, for as we shall see, in the electrolysis of myosin, as well as in that of albumin, alkali-albumin is formed at the cathode. In the anodic region both the watery extract and the sodium chloride extract are much less than in the unelectrolysed muscle. The reason of this is that albumin and globulin are *coagulated* by the products given off at the anode, only a comparatively small amount of acid albumin being formed. The microscopic appearance has already led us to suspect this.

Experiment 32 shews the effect of a very strong current. The neutralisation precipitate in the cathodic portion is increased nearly fourfold; in the anodic region it is more than doubled. In both the coagulable material of the watery extract is much diminished. In the anodic region it has shrunk to less than a third of the normal. Why is the neutralisation precipitate greater in proportion to that in the cathodic region with the stronger currents? The reason, probably, is that the myosin, coagulated by very dilute acid, is dissolved by the stronger acids.

EXPERIMENT 32.—Ox beef. Current of 80 milliampères per square centimetre passed for 30 minutes. Extracted with water for 48 hours.

	Reaction.	Neutralisation Precipitate.	Coagulable Proteids.
Anodic layer.	Acid.	4·1	·8
Cathodic layer.	Alkaline.	6·3	1·3
Unelectrolysed.	Acid.	1·7	2·8

Before we proceed farther with the experiments on muscle, it will be well to detail some observations on the electrolysis of albumin and myosin in simple solutions, for this will help us in the analysis of the more complex process.

Electrolysis of egg-albumin.

Experiment 33.—A current of 14 milliampères (2 milliampères per square centimetre of section) was passed for an hour through undiluted egg-albumin. There was a distinct odour of chlorine at the anode, and this was retained for hours after the completion of the electrolysis. The reaction, which was faintly alkaline at first, was now strongly acid at the positive, strongly alkaline at the negative electrode. Some coagulated albumin had gathered on the anode. The faint yellow tinge of the original solution had disappeared completely in the anodic compartment. The liquid yielded a large neutralisation precipitate, which had all the characters of acid albumin. When this was filtered off, the filtrate still contained a considerable quantity of coagulable albumin. When this too was removed, only the merest trace of a proteid reaction could be obtained from the filtrate.

The liquid in the cathodic compartment contained alkali-albumin in large amount, and coagulable albumin. The unelectrolysed albumin gave only a very slight neutralisation precipitate.

The results of electrolysis of serum albumin were essentially the same as those described.

Electrolysis of Myosin.

Experiment 34.—Rabbit's myosin was dissolved out with 10 per cent solution of common salt. This was used instead of ammonium chloride so as to have a fixed alkali set free at the cathode. The solution is of course a much better conductor than any of the liquids or solids of the animal body. In the first experiment a current of 250 milliampères per square centimetre was sent through the solution. This tremendous current caused an immediate sudden burst of gases at the electrodes, the smell of chlorine being very strong. So much heat was developed that the current had to be reduced at the end of a minute to 100 milliampères per square centimetre, as heat coagulation seemed imminent. It was kept at this reduced strength for two minutes more. The whole of the myosin in the positive compartment was found to be coagulated. It formed a white mass on the electrode, lying in a thin watery liquid. Its reaction was intensely acid. Before the electrolysis the solution was slightly alkaline. The mass was insoluble in distilled water and in 10 per cent sodium chloride. It swelled up and became clear in acetic acid, but did not dissolve in the cold, and dissolved only to a small extent on heating. It was partly soluble in dilute caustic soda in the cold, and readily and completely soluble on heating. From this solution a copious neutralisation precipitate, soluble in excess of dilute acid, could be obtained. The liquid in the anodic compartment gave no neutralisation precipitate, only a faint xanthoproteic reaction, and a slight turbidity on boiling.

The contents of the negative compartment were more fluid than at first, and had acquired a brownish tinge and a strongly alkaline reaction. There was no coagulum. There was a copious precipitate on neutralisation, consisting of alkali-albumin. When this was filtered off, the filtrate gave no coagulum on boiling even when acidulated with acetic acid, and no precipitate on saturation with sodium chloride. It contained only a trace of proteids. It is probable that the heat developed in this experiment hastened the action, as will be seen from

EXPERIMENT 35.—A current of 25 milliampères per square centimetre was passed for 70 minutes through another portion of the same solution. Although the reaction before the electrolysis was slightly alkaline, in four minutes it was neutral in the positive compartment, and in six minutes distinctly acid. In eight minutes from the beginning of the experiment the anode was covered with a white layer of coagulum smelling of chlorine. This layer grew in thickness with the time, just as happens in the electrolysis of blood.

If the amount of change depended wholly upon the quantity of electricity passed, as it would do if the process were a primary electrolytic effect, this current ought to have produced as much change in 18 minutes as the stronger current of Experiment 34 did during the whole time of its passage. So far was this from being the case, that after 70 minutes, the process was still incomplete in the cathodic compartment. There was still much unaltered globulin there, although a considerable amount of alkali-albumin had been formed, the reaction being strongly alkaline. At the anode, the process was almost complete, nearly the whole of the myosin being collected on it as a coagulum possessing the properties already described. A small amount of acid-albumin was in solution, along with a small quantity of unaltered globulin. The reaction was very acid.

This being the state of affairs, the current was now reversed for 40 minutes. If no "irreversible" chemical change had been wrought by the former electrolysis on the proteid substance; if the myosin molecules had been simply rent from their basic mates and transferred unaltered to the anode, as Rollett thought might happen to hæmoglobin; if, in a word, secondary actions played no part; then it was to be expected that the reversal of the current would restore the original state of things. The precipitate which we have called a coagulum, would melt away from the anode, and the negative electrode would clothe itself with a new deposit. Both of these things happened, but both happened to a limited extent only, and under circumstances which shewed that here again the changes were due to secondary actions. The former cathode did indeed, although the reaction of the surrounding liquid continued alkaline, cover itself in time with a thin layer of precipitated myosin. But it was astonishing to find that the whole of this layer, even to its external surface, and the electrode itself, were strongly acid. Although the liquid which bathed the growing coagulum was alkaline, the precipitation only took place where the reaction was acid. Further, it was only the remnant of the myosin left unchanged by the previous electrolysis which was deposited on the new anode. For in other experiments it was found that if the first electrolysis had gone far enough to leave no coagulable material in the cathodic compartment, there was no deposit upon the new anode when the current was reversed. The alkali-albumin could not be precipitated by neutralisation in the immediate neighbourhood of the electrode, for fresh acid would be constantly formed which would redissolve it. The small amount of solution of the deposit on the former anode was certainly not a primary effect. It did not commence at once. The acid reaction of the electrode had first to be set aside by the liberated alkali, and only towards the close of the second electrolysis, when the reaction of the electrode itself, although not that of the outer layers of the mass, was decidedly alkaline, did the deposit begin to be eaten away from the inside. This was well seen when the experiment was done in a glass U-tube. As we have seen, the deposit is partially soluble in dilute alkali in the cold. Its disappearance in the second electrolysis is therefore a secondary effect.

We see now the general way in which the secondary actions affect the proteid constituents. The change in the reaction may itself, apart from actual chemical decomposition, produce important effects on a living tissue, and that not merely in the immediate neighbourhood of the electrodes. It may penetrate deeply between them.

The Antiseptic Action of the Current.

This must be included under the secondary chemical actions, for although removal of the salts will certainly tend to starve the septic organisms, yet it is mainly on the alteration in reaction that the antiseptic influence of the current depends. The antiseptic action of the positive pole, both in preventing sepsis and in removing and restraining an already developed sepsis, is far greater than that of the negative.

EXPERIMENT.—A current of 45 milliampères was passed through 30 c.c. of rabbit's blood for 4 hours. There was no sign of putridity in the liquid of the anodic compartment till 10 days after. That of the negative compartment began to grow putrid in 2½ days. The reaction was acid in the one and alkaline in the other. This is probably only an instance of the general rule that acids are better antiseptics than alkalies. But it is worth remembering that ordinary antiseptic precautions although by no means to be neglected when the anode is introduced into a cavity, are much more necessary when the cathode is the internal electrode.

On some liquids, however, such as bile, the cathode has a distinctly antiseptic effect. Bile containing mucus putrefies very quickly. If it be electrolysed, the liquid around the cathode keeps undecomposed for a considerable time. It may be that this is due to the action on the mucin, for it is well known that if the mucin be precipitated with alcohol, the bile does not decompose even when the alcohol is removed.

Electrolysis has also a deodorising effect on substances already putrid. If putrid bile be electrolysed, not only will the sepsis be stopped, but the smell will disappear. An electrolysis for an hour with a current of 60 milliampères removed the smell completely from 40 c.c. of very putrid bile. The liquid in the cathodic compartment had now the characteristic aromatic smell of fresh bile. That around the anode had in addition a peculiar tan-yard odour. Where there is no porous septum and the products of electrolysis are allowed to mingle freely there is less antiseptic action.

(2) *Removal of Electrolytes.*

We have seen that in muscle the current is carried mainly by the salts. There must, therefore, be constant decomposition of these, and removal of the products to the electrodes. Though diffusion will tend to distribute this loss throughout the whole intrapolar region, and partially to restore it by bringing together again the separated ions, its effect cannot be very great within the limited time of an ordinary electrolysis. In an excised tissue, or in a portion of the intact body in which the circulation is stopped or impaired by spasm of the arterioles, for example, or by clotting of the blood, or in which the stasis of irritation exists, —and it will be seen that there are reasons for supposing that even moderate currents can produce such effects—there must be produced in a very short electrolysis a sensible diminution of the salts. And when we consider how entirely essential this trifling amount of inorganic matter is to every tissue, and that no current can pass without the decomposition of a corresponding quantity of it, it will cease to excite surprise that currents so weak as are sometimes used in electro-therapeutics should produce effects so profound. The primary action of electrolysis is concentrated upon constituents of the tissues which do not amount to more than one per cent of the whole. But that one per cent is essential to life.

If muscle contained ten times as much salts as it does, the electromotive force which produces now a current of 100 milliampères through it, would produce then eight to ten times as much; but the percentage of change would not be increased, there would be no greater disturbance of physiological stability than there is now.

From what part of the circuit are the salts removed? We should expect them to be removed in the first instance from the neighbourhood of the electrodes. But it is easy to calculate the amount of decomposition which a given current must produce in a given time, on the hypothesis that all the electrolytes of muscle are replaced by an equivalent of a single salt, say potassium chloride. In a minute a current of 100 milliampères would decompose about ·0044 gramme of this salt or ·267 gramme in an hour. Taking one per cent of potassium chloride as the "equivalent" of muscle, this would mean that the whole of the salt in 26 grammes would be decomposed in an hour, if the current were kept up to its initial strength. The loss must therefore soon begin to tell upon the intrapolar region, while at the same time it may intensify the secondary actions at the poles. That in addition to the effect of the removal of salts from the intrapolar region, electrolysis may exert upon that region an influence more allied to its polar action, seems certain from the phenomena of internal polarisation. The removal of water by secondary decomposition can scarcely ever be large enough to have an appreciable effect.

EXPERIMENT 36.—Ninety-five grammes of rabbit's muscle were minced and electrolysed in a glass trough, the electrodes being platinum discs equal in area to the section of the trough. A current of 350 milliampères, 35 milliampères per square centimetre, was passed for 30 minutes. The amount of watery extract and of ash was determined in 40 grammes of the anodic half of the muscle, a thin layer next the electrode being first removed, in order to avoid, as far as possible, the inclusion of the electrolytic products in the ash. The following is the comparison with the unelectrolysed muscle:

	Watery Extract.	Ash.
Anodic portion.	1·0 per cent.	·24 per cent.
Unelectrolysed.	5·2 ,,	1·3 ,,

The ash is reduced in a region extending to the middle of the intrapolar region to a sixth, and the total watery extract to a fifth, of the original amount. Now taking potassium chloride again as the "equivalent" of the salts of muscle, we find that a current of 350 milliampères, passing for 30 minutes, would decompose about ·46 gramme. In the 40 grammes of muscle there would be originally, say about ·52 gramme of salts; but at the end of the electrolysis there are only ·096 gramme of ash. ·44 gramme have therefore disappeared from the anodic portion alone. But this is too large a proportion of the loss to fall upon less than half the muscle; and we shall see that the whole of the salts lost by the anodic region are not decomposed by electrolysis, a certain amount being carried towards the cathode by electrical endosmose.

The amount of ash, especially if the whole of the electrolysed tissue be taken, may be much greater than the amount of the undecomposed salts, for it will include the acids and alkalies set free. In this way the effect of electrolysis may be masked.

Experiments 37 and 38 shew that this may happen.

Experiment 37.—The legs of two frogs were taken, the skin stripped off, and the muscles removed. The muscles of the right leg of one frog and the left of the other were stuffed into one of the tubes of skin, the other being slit up to form an outer covering. The other two legs were treated in the same way. They were weighed, and then one of the tubes was electrolysed by a mean current of 20 milliampères for 30 minutes. The total solids and the ash were now determined in each, and although no precautions were taken to prevent some of the electrolytic products being removed on the electrodes, the proportion of ash to solids did not differ, within the limits of error, in the two, the ash being in both cases about 4·4 per cent of the solids. This was not because the amount of decomposition was too small to be appreciated. For a current of 20 milliampères would in 30 minutes decompose about ·026 gramme of potassium chloride, and the whole quantity of ash in the muscle of two legs was ·054 gramme. The cause of the equality was of course the inclusion of the electrolytic products in the ash, only a small amount of the chlorine having escaped.

EXPERIMENT 38.—Everything was the same as in the preceding experiment except that the skin was not included in the determination of the solids and ash, and it was repeatedly wiped during the electrolysis. A distinct difference was now obtained between the electrolysed and the unelectrolysed muscle. In the former the ash was 5·3 per cent of the solids, and in the latter 6·8 per cent. A considerable amount of the electrolytic products had therefore been concentrated in and on the skin. The original muscle in this experiment was much richer in ash than that of Experiment 37. The difference was fully fifty per cent. I do not know whether such a great difference is often found between different samples of frogs.

It may be asked whether in the living body, when the circulation is intact, it is possible for electrolysis to produce sensible differences in the distribution of the salts. I attempted to answer this question by comparing the effect in the frog when a limb is electrolysed, after being ligatured and in the normal condition. But a current of even moderate strength interferes so much with the circulation, and the total quantity of ash in the soft tissue of a frog's leg is so small, that I hesitate to draw any conclusion from the results. The importance of the question is not perhaps so great as might be thought, for it can rarely happen that the circulation is not affected in a part through which a current of considerable density is passing, at least in the neighbourhood of an electrode.

EXPERIMENT 39.—A current of 20 milliampères was passed for 30 minutes through the leg of a pithed frog, the electrodes being strips of platinum foil, one in contact with the anterior and the other with the posterior surface. In four minutes the leg was paralysed, and stimulation caused only twitching of the toes. In twelve minutes the muscles began to feel hard. The circulation in the web was almost completely stopped, although none of the current could pass through it. The vessels were choked with corpuscles. In some parts there were slow oscillatory movements of the blood. There were no reflex movements after the current had been closed for half a minute. At the end of the experiment the toes still responded vigorously to direct stimulation, but the rest of the leg was inexcitable. The amount of solids and ash in the soft tissues of the leg, including the skin, was determined and compared with that of the other leg. No sensible difference was found, the proportion of ash in each case being 4·5 per cent of the solids.

EXPERIMENT 40.—To determine whether this equality was due to the maintenance, in an imperfect manner perhaps, of the circulation in the limb, or to the inclusion of the products of electrolysis in the skin, the foregoing experiment was repeated, but the skin was removed before the analysis was done. The difference in the amount of ash is trifling, only about ·1 per cent reckoned on the weight of the moist muscle. There is no loss of water. On the contrary there is a gain of about 1 per cent in the electrolysed muscles. In fact we have here not pure electrolysis, but the result of a mixed electrolysis and cataphoresis in tissues which are fed in a more or less perfect manner by the circulation.

	Water.	Solids.	Ash.
Electrolysed.	79·1	20·9	1·19, or 5·7 per cent of the total solids.
Unelectrolysed.	78·0	22·06	1·30, or 5·9 per cent of the total solids.

EXPERIMENT 41,—where the influence of the circulation was removed, shews a greater difference. One leg of a frog was ligatured high up in the thigh. A current of 20 milliampères was then passed through it in the usual way for 30 minutes. The ash of the soft tissues, excluding the skin, was found to be 5·1 per cent of the solids in the electrolysed limb, and 6·9 per cent in the unelectrolysed. But the polar changes of reaction extended far into the muscle, and there is little doubt that the actual decomposition of salts exceeded the difference found.

When the circulation is stopped there can be no doubt that the removal of salts may be not only appreciable, but even adequate to the destruction of the tissues. We know that a tissue may be excited first and then destroyed by the diffusion of its salts into distilled water. What effects the removal of salts by electrolysis may have before it leads to the actual death of the tissue, whether excitation by the galvanic current may be related to it in any way; whether the polar changes of conductivity or excitability produced by continued passage of the current can be connected with it, are questions which cannot be discussed here and which perhaps are not ripe for discussion at all. But that removal of a considerable proportion of the salts, even if the removal be temporary, will destroy a tissue is quite sure; and that removal of even a small proportion will affect the nutritive activity is more than probable. This is an action of the current too which may penetrate much farther into the intrapolar region than the secondary actions at the electrodes.

We come now to the third mode of action, which is still less than the second a purely polar action, for it makes itself felt right along the path of the current from electrode to electrode.

(3) *The cataphoric action of the current or Electrical Endosmose.*

This is an action of the current to which but little attention has been paid, in its physiological and therapeutical relations. Yet there can be little doubt, that the great and visible changes which can be produced in this way in the distribution of the liquids of the tissues and the substances dissolved in them, are capable of acting powerfully upon their vitality and nutrition. All the tissues seem to possess the physical structure which renders cataphoresis possible. In living muscle it can be actually observed with the microscope, as the so-called Porret's phenomenon. It has been successfully employed as a means of introducing drugs through the skin. (See a recent paper by Peterson, in the *New York Medical Journal*, April 27, 1889). It certainly plays a part in the diminution of apparent resistance which goes on for some time after the closure of the galvanic current in many electro-therapeutical operations, and which is so striking a feature in the recently introduced gynæcological applications of electricity. I am inclined to think that what gynæcologists have begun to call the "hæmorrhagic" action of the negative pole is, in part at least, a cataphoric effect. There is a transference of liquids to the cathode, and a consequent moistness and turgescence of the parts around it. The smaller the surface of the cathode in proportion to that of the anode, the denser will be the current in its neighbourhood; and as the amount of cataphoresis is proportional to the strength of the current, and therefore the same through every section of the path, the effect with a small cathode in the uterus and a large anode on the skin, will be concentrated in the neighbourhood of the former. The liquids, gathered so to speak from a large catchment area, will pass along an ever narrowing channel and pour themselves out as an increased uterine secretion or hæmorrhage, or condition such a secretion or such a hæmorrhage. In like manner the "hæmostatic" action of the positive pole, although it is certainly due to a large extent to the products of electrolysis set free there, must be partly ascribed to the removal of liquid by cataphoresis.

Again, in all probability, part of the action in the so-called electrolysis of urethral stricture is a softening of the tissue by the flow of liquid to the cathode. For the cathode has been found to be much superior to the anode as the internal pole (Bruce Clarke, *Brit. Med. Jour.*, April 19, 1890). The currents used are so weak that the caustic action of the poles is eliminated, and it is difficult to see what chemical or physical difference there can be which is so important as that produced by cataphoresis.

My experiments have been made (1) on animal liquids and simple solutions of animal substances, through porous septa, (2) on isolated tissues, and (3) on living animals. It is not always easy to say how much of a given effect is due to cataphoresis, and how much to the electrolytic action of the current. Sometimes the two actions conspire, and sometimes they oppose each other; and in general qualitative observations are not enough to separate them. It is necessary to measure the current-strength, to measure the amount of transference of liquids and of the solids dissolved in them, to compare experiments, and to make calculations, and even then it is sometimes possible to arrive only at approximate results. The problem is comparatively simple in the case of liquids separated by a porous septum. There is an immense transference of water from the positive to the negative compartment in the case of all animal liquids and proteid solutions, a smaller proportional transference of salts, and distinct movement of pigments and proteids.

For example, in an hour and a half, with a current of only 5 milliampères mean strength, the volume of a 3·6 per cent hæmoglobin solution had diminished 40 per cent in the positive compartment and increased 35 per cent in the negative, the difference being due to loss in measurement and absorption by the septum.

In a two-hours' electrolysis of bile with a current of 65 milliampères, no less than 92 per cent of the water had disappeared from the anodic compartment, which originally contained 10 c.c. of the liquid. Of course only a small proportion of this loss was due to decomposition of the water. Similar results are got in liquids rich in proteids, but here the coagulation at the anode modifies the process. A long-continued electrolysis, however, dries the anodic coagulum in its turn by cataphoresis; and this process certainly has a share in the *physical* effects of the positive pole.

In excised tissues the process, as we have said, is more complex. Thus in one experiment where 70 grammes of rabbit's muscle were electrolysed for 30 minutes by a current of 350 milliampères, the water in the cathodic half was found to exceed that in the anodic half in the proportion of 48 to 31, and the salts in the proportion of 48 to 23. But here the direct decomposition of the salts was mixed up with the change in their distribution by cataphoresis.

In the living animal there is a third factor which must powerfully affect the results, the physiological factor namely. For example, in electrolysing the uterus of a rabbit, with a hollow metallic electrode passed up the vagina, I have noticed that when the hollow electrode is the cathode, fluid is forced through it, while this does not happen when the intravaginal electrode is the anode. Is this due to increased secretion caused by the stimulation of the cathode alone or is the cataphoric action of the current in play? I merely state the problem to show how difficult the analysis is, without going into the reasons which have led me to believe that the physical action has at least an important share in processes of this kind. The whole subject would be better discussed elsewhere in connection with the practical application of electrolysis in disease.

(4) *Local Increase of Temperature produced by the Current.*

As to *the local increase of temperature* which I have distinguished as the fourth chemico-physical mode of action of the current it is not necessary to say much here. In dead tissues, and practically in tissues isolated by excision or ligature, it is a purely physical problem to calculate the quantity of heat evolved by a given current in a given time. It varies directly as the square of the current, the first power of the resistance and the time, when the energy of the current is all transformed into heat. When part of the energy is employed in chemical decomposition, as is always the case in electrolysis of animal tissues, the heat will be less by a corresponding amount. The rise of temperature produced in an isolated tissue by a given current in a given time, chemical decomposition being left out of account, will vary directly as its resistance, inversely as its capacity for heat, and inversely as the loss by radiation, conduction, &c. When the stationary temperature has once been attained, the heat loss will cease to have any influence, and the excess of temperature will depend only on resistance and capacity. For example, the specific heat of nerve and muscle being taken as about equal, the capacity of a given length of the sciatic nerve of a frog will be to that of the same length of the gastrocnemius muscle approximately as their mean sections. Their resistances will bear the same proportion. The rise of temperature will therefore be inversely as the square of the mean section for a given current strength. Thus a current which may not raise the temperature of the muscle by more than a fraction of a degree, may shrivel up the nerve.

EXPERIMENT 42.—Two troughs of the same length, but with cross sections, in the proportion of 1 to 20 were filled with minced rabbit's muscle, and electrolysed in series by a current of 400 milliampères. The temperature in the narrow trough rose in a quarter of an hour to 70° C., the temperature of the room being only 14°, while the other was scarcely affected. When the troughs were arranged in parallel arc, the same E. M. F. being used, there was no appreciable rise of temperature in either.

This is, of course, only an illustration of a well-known law.

EXPERIMENT 43 shews that it is possible to obtain well-marked differences of temperature in a continuous mass of tissue by electrolysing it. 45 grammes of rabbit's muscle were placed in a hollow triangular prism of glass, and a current of 200 milliampères passed through it for 40 minutes from apex to base. The temperature at the apex rose rapidly, that at the base more slowly, until at the end of the experiment the former was 54° C. above that of the air, and the latter 16° C. above it. There was thus a difference of 38° C., although the distance between the two electrodes was only 7 centimetres.

In the living body the circulation will, of course, tend to prevent the local rise of temperature, but, notwithstanding this, we know that marked local differences, even in the blood stream itself, exist. The resistance of the tissues is generally so great, compared with the internal resistance of the battery, that nearly the whole heat production must be between the electrodes. When one of the electrodes is small compared with the other, the heat production must be correspondingly increased in its neighbourhood. We often hear of the immense production of heat by a gland like the liver. But in an ordinary gynecological electrolysis the chemical action of a battery of 30 or 40 volts working through a resistance of 300 ohms may be concentrated within two or three hundred grammes of tissue. Is it possible that no appreciable change of local temperature is produced?

EXPERIMENT 44.—In a cat just dead a thermometer was introduced into the vagina by an incision. The negative electrode was put into the vagina by the natural opening, and the positive, which was much the larger of the two, was placed over the uterus. A current of 35 milliampères was passed, and in three minutes the temperature had risen from 25°·4 to 27°·2. (The cat had been used for a previous experiment, which accounts for the low temperature). When the current was opened the temperature fell rapidly to 25°·5. The current was now closed again in the reverse direction, the intravaginal electrode being now the anode. In four minutes the temperature was 26°·3, and remained stationary at that during the passage of the current. When this was stopped it again fell rapidly to a little below its former height, as the animal was still cooling.

Here, of course, the circulation was stopped, and it was still necessary to see whether in the living animal any change could be observed.

EXPERIMENT 45.—A rabbit was put under chloroform, and the skin of the abdomen shaved. A large disc electrode, covered with cotton wool, moistened with warm normal saline, was adjusted to the shaved surface, while a tubular electrode, carrying a thermometer in its lumen, was introduced into the vagina. A current of 30 milliampères was passed, the intravaginal electrode being first the anode. Then another series of observations was taken with the cathode in the vagina.

Anode in Vagina.

Time.	Temperature.	Remarks.	Time.	Temperature.	Remarks.
4.29	38°·8	Current now closed.	4.57	38°·4	
4.29′ 30″	39°·25		4.58	38°·7	
4.37	39°·21		5.	38°·92	
4.40	39°·21	Curr'nt opened	5.1	39°·	
4.40′ 30″	39°·1		5.2	39°·	
4.42	38°·7		5.3	39°·	Curr'nt opened
4.44	38°·55		5.4	38°·6	
4.47	38°·4		5.5	38°·5	
4.54	38°·2		5.8	38°·32	
4.56	38°·2	Current closed	5.10	38°·2	

Cathode in Vagina.

Time.	Temperature.	Remarks.
5.26	37°·5	Current closed.
5.29	37°·9	
5.31	38°·	
5.33	37°·95	
5.34	37°·9	
5.35	37°·7	Current opened.
5.37	37°·55	
5.39	37°·45	

The progressive, although slow, cooling of the animal, although it was protected as far as possible by cotton wool, introduces a disturbing factor. Yet there can be no doubt that there is a prompt and appreciable rise of temperature when the current is passing. The limit is soon reached, and the range is not very wide, for the higher the temperature of a part may be, the more heat does it part with to blood cooler than itself. And it must be remembered that the development of heat is proportional to the square of the current. A current of 150 milliampères, which is within therapeutical limits, would produce a heating effect 25 times as great as the 30 milliampères of our experiment, under similar conditions. We must not suppose, however, that the extreme rise of temperature, which was ·9° in that experiment, would be 25 times as great with five times the current. While the circulation is intact, nothing like the theoretical limit can be reached. On the other hand, we must remember that the temperature in the vagina is only a rough test of the temperature in tissue elements where the action is most intense; and it is not at all impossible that local heat coagulation of some of the proteids of the tissues may be one result of electrolysis by a strong current. This would mean local death, and consequent absorption. But a circumscribed elevation of temperature stopping short of this, a local fever so to speak, will probably while it lasts lead to increased metabolism. If it lasts long it may even prepare the way for fatty degeneration. In any case it will certainly increase the chemical action of the electrolytic products; which is already greatly favoured by the normal bodily temperature. It may be asked whether the rise of temperature observed in the vagina is not due to a general rise of the temperature of the blood caused by the contraction of the abdominal muscles and the uterus, intestines and other abdominal and pelvic organs. The constant current does excite powerful and continued contraction of the uterus, the abdominal walls are tense, and peristalsis of the intestines is very active. But the rise of temperature in the vagina is not due to any of these things; it is not due to a general rise in the temperature of the blood; for the temperature of the rectum is not affected, nor is that of the mouth. The heating action of the current tends to reduce the resistance, but it can scarcely ever be large enough, in the living body, to account for more than a small part of the progressive increase in the current so often observed.

Electrolysis in Therapeutics.

I do not propose here to discuss in all its bearings the relation of these results to the therapeutical use of strong voltaic currents. The inquiry is far from being complete, even on its physical and chemical side; and a mass of physiological and pathological observation is required before we can fully understand how the physiological effects of the current modify the physical, and the physical the physiological, and how both affect the processes of disease. But I should like to connect the subject, before leaving it, with its practical applications by a link or two of suggestion.

In the first place it is clear that the polar effects are to be sharply separated from those parts of the electrolytic action which influence especially the intrapolar region. They are, in their action on the body, the most purely physical of all the effects of the current; they are the least indebted to slow physiological processes induced by them and continuing after them. The polar action can only reach tissues at a comparatively small distance from the electrodes. It is therefore specially suited for skin surfaces, and the surfaces of accessible cavities. If the epidermis is unbroken it is unlikely that the polar action will reach beneath the subcutaneous tissue. But there will be a better chance of its doing so if the epidermis is soaked with a strong solution of salt. Wherever an intense polar action is required, the tissues should have as much salts in them as possible; for the more intense the current the more of the electrolytic products will be given off at the electrodes. Wherever the active pole may be the indication is the same.

As a rule it has been found necessary, in order that the polar action may be effectual in the case of a subcutaneous or submucous structure, to get the electrode into the structure by puncture. In the treatment of naevi, Duncan not only punctures, but moves the positive electrode about in the tissue, relying entirely upon the secondary electrolytic action, aided perhaps to some extent by the mechanical effect. Here destruction of the tissue is what is aimed at, and the polar action is admirably suited for this. The attempt to treat a naevus by electrolysis without puncture, which is still sometimes made, is based upon an inaccurate idea of the distribution of the polar action. Since it is most intense at the surface of the electrodes, whatever mischief is done to the naevus must be accompanied by greater mischief to the skin if there is no puncture. It is indeed possible with a strong current to destroy a naevus through the unbroken skin, but not without injuring the skin. It is possible to stop the circulation in the carotid artery of a rabbit without puncture by applying metallic electrodes directly to the artery, but it needs a very strong current, and the artery itself must first be baked and cauterised. I have passed a current of 50 milliampères by well insulated electrodes through the exposed carotid for ten minutes without producing a single symptom of disturbance to the circulation, although chlorine was coming freely off from the anode. Indeed, of all tumours, a vascular tumour is the best suited to the polar action and the least amenable to the other actions of the current. It is hopeless to attempt to starve it by the removal of its salts, so long as the circulation goes on; its structure does not lend itself to a very active cataphoresis, and in any case the supply of fluid is too free for this to have a sensible effect, and heat coagulation is out of the question. On the other hand, its vascularity makes it a good conductor. There is plenty of salts in it to yield decomposition products at the electrodes, and the coagulation of the blood by the anode prevents these products from being carried uselessly away.

A superficial new growth, an epithelioma, for example, which does not infiltrate deeply the subcutaneous tissues, would be another condition suitable for the polar action of the current. When I say this, I am not advocating such a line of treatment. It may be highly improper, from a surgical point of view, or it may be the reverse. What I say is, if it is desired to destroy such an epithelioma by electrolysis, the polar action of the current is that which is best suited to destroy it; and here again, the more salts which can be got into the tissue the better. If, on the other hand, we have a cancer spreading far beneath the skin, still more, if we have a cancer in an internal organ and far from a mucous surface, the polar action cannot reach it without puncture.

The kind of tissue most likely to be influenced by the removal of salts by electrolysis, and by the cataphoric action of the current rather than by the polar action, would be a tissue without a very rich vascular supply or what comes to the same thing with a vascular supply liable to be interrupted by the physiological effects of the current. If at the same time the tissue required an unusually large amount of salts for its proper nutrition the likelihood of its being influenced in this way would be increased. A uterine fibroid is probably a fairly good example of a structure fulfilling the first condition, and all inflammatory exudations are said to fulfil the second. (Gorup-Besanez, *Physiol. Chemie*, III., p. 87). Hence, although it is true that at any rate in an intra-mural and still more in a subperitoneal fibroid or in a cellulitic induration, there can be no polar action without puncture, it is not correct to say, as has been said, that there can be no electrolytic effect. There is a very great current-density in an ordinary uterine electrolysis in the neighbourhood of the internal electrode. In many cases, if not in all, the uterus is firmly contracted during the passage of the current, and even for many hours after the patient may have bearing down pains. In this state of things the circulation in the fibroid is not likely to be very active. The vasomotor arrangements are far less perfect in pathological growths than in normal tissues, and the contracted uterus may feed itself while the tumour is starved of its proteid nutriment, and at the same time robbed of its salts, and while the normal distribution of its liquids is deranged. The richness of inflammatory products in salts, and especially in good conducting salts like sodium chloride, all the more if coupled with a special need for them and an imperfect blood supply, will tell in two ways: loss of the salts will be more serious and less easily repaired than in the normal tissue, and at the same time the resistance will be less, and the share of the current correspondingly greater. In the same way a cancer, if it really contains an excess of salts, as is alleged, would to a certain extent short-circuit the current from the normal tissues in which it lies. [A number of the experiments were done in the Physiological Laboratory of Cambridge University.]

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The Action of Various Stimuli on Non-Striped Muscle.

BY

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(PLATE I.)

THIS contribution contains the results of an investigation undertaken to ascertain the action of various stimuli on the musculature of the stomach of the Frog.

The structure of the muscle contained in the walls of the Frog's stomach is that of the non-striated variety. Much more work has been done recently on striated than on non-striated muscle, hence the results of the former are much more widely known, so that it may not be out of place, before giving my own results, to give a short summary of the results of other observers.

Engelmann found that the ureter is capable of undergoing peristaltic movements—apart from any nervous influence—on stimulation, which persist after the cessation of the stimulus.

The results which Van Braam-Houckgeest and Ranvier, working on the alimentary canal of the Rabbit and Frog respectively, obtained are much alike. They are as follows:—

1. Certain parts of the alimentary canal, the duodenum and jejunum, are the centres around which peristaltic movements take place; but all parts, providing they be in a proper medium, are capable of undergoing rhythmical contraction.
2. These rhythmical contractions are spontaneous, and persist for a considerable time, and are capable of being modified by various stimuli.
3. The muscular coating of the stomach of Frogs, according to Ranvier, consists of only a circular layer of fibres;

these are the active agent in causing contractions, and not the muscularis mucosæ.

I propose now to relate my own observations, comparing them with those of Ranvier, and then to give an account of the action of various drugs on the musculature of the Frog's stomach.

1. *Structure of the Stomach.*—It is unnecessary to go into detail here, my remarks being chiefly confined to the musculature. From without inwards we have the following layers:—

(*a*) Muscular layers composed of *external longitudinal* and internal circular layers.

(*b*) Sub-mucous or connective tissue layer.

(*c*) Mucous membrane with muscularis mucosæ.

With regard to the layer (*a*), there is certainly a longitudinal layer of smooth muscle covering the circular fibres, but by no means of the same thickness. It is present in all the sections I have cut; it is thin at the cardiac extremity, but gradually increases as it gets towards, and is thickest at, the pylorus. The circular layer appears of about equal thicknesses at the two extremities of the stomach, and is thickest in the middle, the proportion between the two layers (longitudinal to circular) being at the cardia as 1 : 5, at the middle as 1 : 8, and at the pylorus as 2 : 5 respectively. Between these two layers the plexus of Auerbach is placed, and hence reagents will have to soak through the longitudinal layer before they can have any influence on the ganglia. Ranvier, who says there is only a circular layer, necessarily places this plexus simply beneath the peritoneal covering; hence he gave this as a reason why the stomach should be so irritable. It remains, however, to be proved whether this nerve plexus has any effect on the rhythmical contractions. Certainly, the experiments of Engelmann on the ureter, and various observers on the heart-apex of the Frog, throw doubt on its function. What the primary cause is of the rhythm in the stomach or the heart, is yet undecided. In the case of the heart-apex, one of the main factors is the pressure exerted by the circulating fluid. By increasing the pressure in a heart-apex or heart preparation which has stopped beating, the contractions can be caused to recommence. This is not borne out in the stomach, however; for although it can well be compared with the heart, it will contract equally as well when the pressure is as great inside as out; in fact, all the results recorded here were got by simply allowing the stomach to soak in the fluid, the pressure on the inside and outside thus being equal.

What, then, will the effect of these antagonistic layers on the lever be? If we add the ratios of each part of the stomach together, we shall have roughly the following:—Circular: longitudinal :: 18 : 4, or as 4½ : 1. The circular layer thus greatly preponderates, so that we may leave the longitudinal out in our calculations. As to the effect of the muscularis mucosæ, I may add that since each layer is about equal in thickness it will be *nil*; so that the essential element in contraction is the circular layer, and this is borne out by the manner in which the stomach reacts to drugs, and by a comparison with striated muscle.

Method of Experiment.—The experiments were done with an apparatus resembling Wild's[1] modification of Brunton and Cash's[2] (see Fig 1). This consists of a glass cylinder (B), made by removing the bottom of a 2-ounce phial, and closing the mouth with a cork. The latter was perforated by a thin copper wire, the upper end being bent on itself into the form of a hook,

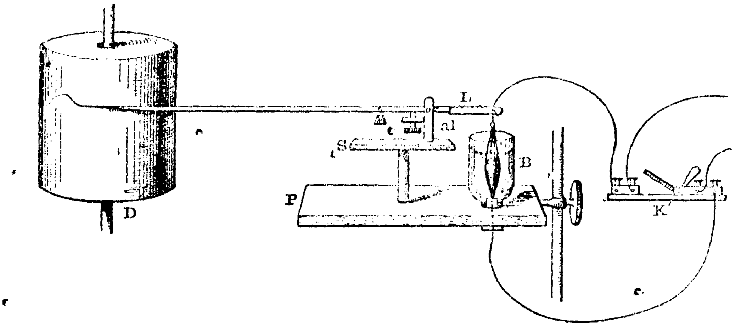

Fig. 1.—Apparatus used. For the skeletal muscle the excised stomach of the Frog was substituted.

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[1] *British Medical Journal*, September 13, 1887.

[2] *Phil. Trans.*, 1884.

which was pushed through the pyloric extremity, and then pressed tight so as to clamp this end perfectly. Another long thin copper wire was passed through the cardiac end, and similarly clamped tight, its upper end being screwed to the short end of the lever (L). By this means a current of electricity could be sent through the preparation. The cork was then fitted to the neck, and the whole inverted, the neck of the bottle passing through a hole in the platform (P), and the phial then filled with Ringer's fluid. The long arm of the lever was composed of straw, measuring 25 inches, the short arm being 1½ inch. By means of a counterpoise (*cl*) on the latter, the work of the stomach could be reduced to a minimum, and the end of the lever was furnished with a small piece of stiff paper cut to a point, thus acting as a writing style on a blackened drum (D). This drum, owing to the slowness with which the stomach contracts, was driven by the axle-rod of Hawksley's recording apparatus placed on its side, and thus I was able to get a speed of ⅞ inch, or just over two centimetres per five minutes. The platform was fitted to a brass up-right, along which it was able to move, and capable of being fixed at any height by means of a screw. This upright was fitted into a stand, and by means of a tangent screw it could be rotated through one-sixth of a circle. By this means the lever point could be made to write with the smallest possible amount of friction.

With this apparatus the first few experiments were performed; but in pipetting off the solutions, when they required changing, the air caused the stomach to contract very irregularly, and it was necessary to obviate this effect. This was accomplished by placing a funnel, held in a retort-holder, above the cylinder, its lower end being connected by means of a caoutchouc joint, with a glass tube which dipped in the fluid contained in the cylinder. This caoutchouc joint was clamped, and the funnel filled with a solution of the required reagent. The cork closing the neck of the bottle was also perforated by a hole, through which passed a glass tube, having its lower end closed by a clamped caoutchouc tube. By this means the apparatus could be emptied and refilled in about five seconds, and this made the effect of the air inappreciable. With it I obtained fairly good contractions; it is an easy and quick method of getting results, and one subjecting the stomach to very little irritation.

In *preparing the stomach for experiment* the abdomen of the Frog (*Rana temporaria*) was opened, in all cases, under normal saline. The only period in which the stomach was exposed to air was during the fitting of the cork into the neck of the bottle, the hooked wire and clamped glass tube being fitted into the cork before the stomach was touched. It is necessary, in separating the stomach, to take sufficient of the duodenum and œsophagus to give room for the forceps to rest, great care being taken not to injure the walls of the stomach; the hooks, holding it in position, of course are placed only in stomach structure.

Washing out the Contents of the Stomach.—Ranvier lays stress on this. It is, however, not always possible or necessary, because in some cases, when the stomach contained particles of food, I got as good results as when it was washed out; still, on the whole, it is the better plan. In doing so, it is well not to use too much force, or it may be spoiled through over-distension.

Normal Tracing (Pl. I. fig. 1).—The normal tracing here described is that which I obtained with Ringer's fluid, because this solution was used in all the experiments, being better than either blood or normal saline, which will be described later.

This tracing consists of spontaneous contractions indicated by the rise and fall of the lever. At first, while the stomach is in an irritable condition, due to the manipulation consequent on putting it up, the contractions were small and irregular; after some time they became larger and more regular, and finally the stomach passed into a condition where the contractions became longer, weaker, and less rapid. They are essentially like those of striated muscle, differing only in the greater length of the latent period, slower ascent and descent, with longer elastic vibration. In addition there is always a primary continuous fall of the lever, lasting from five to ten minutes, which I can only ascribe to the irritation consequent on putting up. It cannot be due to the contraction of the longitudinal layer, for this would cause a rise, but is most probably due to the spasmodic overaction of the circular layer, because it disappears in a short time, and then the lever describes an almost horizontal line. The rise or fall is caused by the elongation or contraction respectively of the circular layer, as will be evident from what has been said above about the structure of the stomach and the kind of lever used.

The Frogs also vary somewhat. I found the contractions better in strong adult male and breeding female Frogs. During the months of August and September the contractions obtained were very small and feeble.

Normal Saline.—This was tried at first. The results with this are very variable, sometimes no spontaneous contractions being present. When they are, they present the same general characters as with Ringer's solution, but are not so strong; they are irregular in rhythm, and only persist for about an hour at the longest.

Diluted Blood (fig. 2).—This is better than normal saline, but on the whole not so good as Ringer; only in one experiment did I get a better contraction than in Ringer. The solution is by no means so convenient to work with; it is made by adding two parts normal saline to one part blood, and requires to be fresh when used.

Mechanical Stimuli.—On pinching the stomach *in situ*, a circular constriction opposite the point stimulated was produced, and also a small one longitudinally. The former is due to the action of the circular, the latter to the longitudinal layer of muscles. Ranvier, who got the same results, assigns no reason for the latter reaction, since he says only circular fibres are present. The muscularis mucosæ can take no part in the reaction, for its layers, longitudinal and circular, are of equal thickness, and so thin as compared with the external muscular coat. Of the different parts of the stomach, the pyloric region is that which produces the largest contractions, though all parts contract.

Thermal Stimuli.—With the abdomen opened and the intestines *in situ* and in normal saline, large movements took place at a moderately quick rate at the temperature of 16°·5 C. The whole of the movements appeared to be situated round the duodenum as a centre, the whole of the remaining portion of the alimentary canal being as it were moved round this centre. They were kept up for upwards of six to eight hours. A temperature of 20° C. increased their rapidity, but above this they diminished in frequency, and stopped at a temperature above 26° C.

When put in the apparatus with lever, &c., the rhythmical contractions increase in frequency and size up to 18° C. This is, however, variable, some stomachs contracting at 20° C. A temperature of 28° C. abolishes them, the stomach being in a state of contraction (rigor). Ranvier says the high temperature limit is 32° C.; otherwise his results are similar to the above. The contraction at 28° C. appears to be a true "heat-rigor," and differs from the so-called "cold-rigor," in not being able to recommence contracting upon the temperature being reduced.

In all the following experiments the stomach was allowed to contract about fifteen to twenty minutes in Ringer's fluid so as to get a normal tracing, the current was then sent through or the drug added, so that the comparison between the two might be more correct, as differences in the normal tracing, in one way or the other, sometimes took place. At times the reagent was only added when the stomach had stopped contracting, in order to see the result.

Faradaic Stimuli.—With a weak current the contractions are increased in force and the frequency diminished, but the rhythm remains regular; a current of medium intensity caused a slight rise of the lever, which remains so; while one of maximum strength sent the stomach into a state resembling tetanus, the wave-like contractions being abolished. Thus the effect produced simply depends on the strength of current used.

Galvanic Stimuli.—With one Daniell's cell (Fig. 26) the spontaneous contractions were made stronger, slightly slowed, and the rhythm more regular, the form of the curve being broader at the apex. The contractions continued regularly for many hours, and with but slightly diminished vigour, the results seeming to be rather of a tonic character. There was no essential difference whether the current was an ascending (from the pylorus to the cardiac end) one or descending, or whether a make or break. The effect of the current seems to last a long time after its withdrawal. In addition it has the power of initiating spontaneous contraction (Fig. 5). A slight but continuous relaxation of the stomach is caused just like the action of dilute acids on striated muscle. With three Daniell cells the contractions were still present, no difference being noticeable between this and the tracing obtained with a weaker current (one cell). This differs from the effect of a constant current on the heart, which was shown by V. Ziemssen to double the number of beats when a strong current was passed through it and by Ludwig and Hoffa, to bring about a condition resembling tetanus, when a very strong current was used.

Chemical Stimuli—Lactic Acid.—With a solution of $\frac{1}{10000}$ (Fig. 3), the spontaneous contractions were not altered in force, but were more frequent and rhythmical; they soon disappear, becoming very small in about five minutes, and almost imperceptible in fifteen. The lever rises slightly at first, showing relaxation of the stomach (circular layer), and then gradually falls (contracture), thus differing from the effect in stronger solutions, in which it agrees with the experiments of Ringer on the heart-apex, who found that acids with muscarin and pilocarpin cause a state of relaxation of the heart, while alkalies with veratria and digitalin cause a state of contraction. The above results were obtained in the winter season, at the latter end of January and beginning of February. In October, when the weather was not so cold, a solution of the same strength had less paralytic effect, the spontaneous contractions only being reduced to small rhythmic ones in fifteen minutes, and on adding Ringer's solution, the contractions became stronger, and persisted for half an hour; otherwise the effect on the lever and stomach was the same.

With solutions of $\frac{1}{5000}$ (Fig. 4) the spontaneous contractions were stopped immediately; the stomach was suddenly but slightly relaxed, but afterwards passed into a state of gradual but sustained relaxation. When in this condition, a solution of $\frac{1}{1000}$ caused the relaxation to become more rapid, and next day the stomach was in a soft relaxed condition. A solution of $\frac{1}{1000}$ (Fig. 6) accentuated the above features.

In all the above, while under the action of the reagent, the stomach becomes bent on itself, and thrown into circular folds, which begin at the pyloric end and go upwards.

Thus lactic acid is a strong poison to smooth muscle, even in dilute solutions, the rapidity of its action varying as the strength of solution. It causes a relaxation of the muscle, and agrees in these points with its action on striated muscle, the heart, and the smooth muscle of blood-vessels. The stimulating effect, which the weakest solution, $\frac{1}{10000}$, has, in accord with a theory recently set forth, that all drugs, in a certain strength of solution, have a stimulating action, although they are ordinarily classed as strong muscle poisons.

Sodium Hydrate.—Solutions of $\frac{1}{21000}$ (Fig. 7) made the spontaneous contractions stronger, more frequent, and more regular in rhythm. The form of a single curve is as follows:—It commences with a sudden rise, which becomes slower as it reaches the summit of the curve, which is more pointed; then the descent begins, suddenly at first, but more slowly as it approaches the base line. As the action of the drug continues the contractions become weaker, the top more rounded, and the latent period longer; the contractions persist for hours, with gradually increasing contracture of stomach, which is, the following morning, in a strongly contracted and hard condition.

In strengths of $\frac{1}{18000}$ the same reaction takes place, but the spontaneous contractions are much smaller. In certain stomachs which did not contract, spontaneous contractions were produced, small at first, becoming larger and flat-topped, but finally weak again.

With a strength of $\frac{1}{12000}$ (Fig. 8) the spontaneous contractions are abolished; in some cases at once, in others after about five minutes' action.

A solution of lactic acid, $\frac{1}{10000}$, was added to a stomach which had been half an hour in sodium hydrate $\frac{1}{12000}$; it causes a sharp rise, at once distinct from the sodium hydrate tracing. This rise presented a few contractions, which soon disappeared, and then the tracing became horizontal. After fifteen minutes the solution was changed to sodic hydrate ($\frac{1}{12000}$), and then the normal action of this substance was brought into play, a moderately quick—but not so quick as that of the lactic—fall of the lever taking place, which became continuous. Thus the effect of sodium hydrate, $\frac{1}{12000}$ is antagonistic to that of the lactic acid, $\frac{1}{10000}$, and they both prevent the spontaneous contractions taking place.

Briefly, sodic hydrate in weak solutions acts as a stimulant, but in strong solutions it paralyses, the stomach after a long period being in a state of *rigor mortis*. Lactic acid also in weak solutions stimulates, but quickly—indeed, very quickly—paralyses. In strong solutions it paralyses at once, and in all the stomach is in a relaxed condition.

The above results are essentially the same as those of Ringer on the heart-apex, with the exception that, using levers of similar orders, he got a fall with lactic when I got a rise, and the reverse with sodium hydrate. This, however, is only apparently so, for in reality, when we consider that it is the circular fibres of the stomach which cause the lever to move, and that both ends of the stomach are clamped and its cavity filled with fluid, we shall see that a contraction of the stomach will not cause a rise of the lever, but a fall, and relaxation of the stomach the reverse. This is, in fact, one proof of the circular layer being the active agent in these experiments.

Sodium Nitrite.—With a strength of $\frac{1}{10000}$ (Fig. 9), the spontaneous contractions increase materially in force but not in frequency, and their form was altered. The ascent was quick, the top pointed, the descent showing at first a sudden fall, becoming less rapid near the base, and the latent period gradually becomes longer. The after-effect of Ringer's solution makes the top broader. The spontaneous contractions disappear less quickly than with sodium hydrate or lactic acid. The stomach is gradually but continuously contracted. Strengths of $\frac{1}{1000}$ caused the top of the wave to become rounder, but paralysis takes place gradually from the first, the spontaneous contractions being almost gone in eighty minutes. On the addition of Ringer's solution (Fig. 13), they reappear, are well marked, but weaker.

Potassium Nitrite.—With solutions containing $\frac{1}{50000}$ the contractions are increased in force, the frequency is unaltered, and the rhythm is irregular. On changing to Ringer's fluid, they are made larger and more regular, but there is no definite form of contraction-wave. The stomach undergoes gradual but very slight contraction.

A strength of $\frac{1}{10000}$ stops the spontaneous contractions in ten minutes. They recommence on adding Ringer's fluid, at first being irregular both in force and rhythm, but afterwards regular but weak, and they persist a long time. Hence it would appear that $\frac{1}{50000}$ acts as a stimulant to, while $\frac{1}{10000}$ poisons smooth muscle. All cause a slight amount of contracture.

Quinine Sulphate.—In solutions of $\frac{1}{1000}$ (Fig. 14) quinine acts best; weaker solutions have little if any definite action; stronger solutions or prolonged action of $\frac{1}{1000}$ causes the spontaneous contractions to disappear, and the stomach to pass into a state of rigor. With $\frac{1}{1000}$ (Fig. 15) the spontaneous contractions are initiated when absent. If present they are increased in force, lessened in frequency, and increased in rhythm. After a time they diminish and gradually become obliterated. The rise of the lever in Fig. 15 does not occur normally, but the reverse generally takes place, viz., contracture or fall of lever.

Wild[1] got the same state of contracture on the involuntary muscle of the œsophagus of the Frog. He comes to the conclusion from experiments on striated and non-striated muscle (in the vessels, heart, and œsophagus), that quinine acts essentially the same on all forms, stimulating in small doses, paralysing in large or continued action of small, and causing a state of *rigor mortis* after the action of very large or prolonged action of large doses. The results are very similar to those on the stomach.

Quinine is said not to excite uterine contractions, but to stimulate them when present, and to cause tonic contractions of the uterus. Some of the experiments on the stomach would lead one to suppose that it will initiate spontaneous contractions.

Strychnin Sulphate.—Solutions containing $\frac{1}{72000}$ and $\frac{1}{72000}$ gave no definite result as to the spontaneous contractions. The effect on the lever (rise or fall) was also *nil*. With $\frac{1}{20000}$ (Fig. 16) the spontaneous contractions were stopped in thirty minutes. Their force is slightly increased. The stomach is also very slightly contracted at the end of the experiment. With $\frac{1}{15000}$ the spontaneous contractions were quickly arrested, and the stomach rapidly passes into a state of contracture, which was stopped by adding Ringer's solution.

Strychnin sulphate thus acts as a stimulant in weak solutions at first, but quickly poisons. In strong solutions this stimulant effect is absent.

Pilocarpin Hydrochlorate.—Solutions of $\frac{1}{10000}$ (Fig. 17) caused the spontaneous contractions to be slightly increased in strength, though they continue for a long time, *i.e.*, there is little poisoning effect. The rhythm is regular, and it is able to initiate spontaneous contractions. The form is characteristic; the accent is moderately quick, the summit round, and the descent slower than the ascent. In strengths of $\frac{1}{5000}$ the contraction form was well shown, though weaker. The addition of

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[1] *Brit. Med. Jour.*, September 3, 1887.

Ringer's solution brings out this difference better. With $\frac{1}{1000}$ (Fig. 18) the form of the spontaneous contractions was best marked, the ascent being moderately quick, and the descent slower; the form is altered on adding Ringer's fluid, but returns again on adding pilocarpin. Even in this strength the poisoning effect is very little, and the stomach undergoes slow but gradual contracture. In striated muscle it causes fibrillar contractions, by irritating the motor nerve endings (Brunton).

Atropin Sulphate.—In solutions of $\frac{1}{10000}$ (Fig. 24) the spontaneous contractions were lessened in force, the frequency not altered, but the rhythm made more regular. There was no definite form of contraction-wave, but it had on it a number of irregularities. There was very little paralysis, the spontaneous contraction going on after two hours; neither was the stomach in a state of contracture or relaxation.

Solutions of $\frac{1}{5000}$ had the same general effects as the above. Thus this does not agree with the researches of Szpilmann and Luchsinger, who say that atropine acts as a specific poison for smooth muscle. They found that after the action of atropin stimulation of the peripheral end of the vagus will cause contraction of the striped muscle in the œsophagus, but not of the smooth variety, although both forms of muscular tissue respond to direct stimulation. W. Stirling points out that this is doubtful, because there are no end plates in smooth muscle, so that the link between the nerve fibrils and the contractile substance is probably different in the the two cases.

Veratrin Sulphate.—Solutions of $\frac{1}{20000}$ made the spontaneous contractions stronger, more frequent, and rhythmical. The stomach undergoes at first a dilatation, then a very gradual contracture. With strengths of $\frac{1}{10000}$ the spontaneous contractions were just like a monocrotic pulse-wave (Fig. 25); they remained so for about twelve contractions, and then began gradually to show a wave like that of the radial artery, and they are present for some time. The stomach in this and the rest was similar to the above in a state of contracture.

Strengths of $\frac{1}{5000}$ (Fig. 11) caused the monocrotic waves to be well developed, and on adding Ringer's solution for a short time the pulse-like tracing reappeared. In this case they were accentuated by again adding veratria. With $\frac{1}{1000}$ the spontaneous contractions stopped, but reappeared in an accentuated form on adding Ringer.

Digitalin (Fig. 20).—With a solution of $\frac{1}{30000}$ the spontaneous contractions were increased in strength, lessened in frequency, and the rhythm remained regular. The contractions are not unlike those of a radial pulse tracing, with gradually decreasing tension. The stomach also undergoes slight contracture.

Ringer has pointed out that veratrin and digitalin agree with alkalies in causing the muscle of the heart to pass into a state of contracture, while acids, with muscarin and pilocarpin, cause relaxation. It will be seen that with veratrin and digitalin a similar action takes place with the non-striated muscle of the stomach; whether it is due to their action on the nervous or muscular tissue remains to be proved. According to most observers, in the heart at least it is said to act on muscle. On the stomach, however, muscarin and pilocarpin cause contraction, not dilation.

Muscarin Nitrate.—With strengths of $\frac{1}{15000}$ (Fig. 21) the spontaneous contractions are increased in force and unaltered in frequency and rhythm, which last remains good. The form is also altered—a sudden ascent, then a pointed apex; then the descent, at first sudden, but becoming slower, and drawn out as it approaches the base (Fig. 22). It is very characteristic. With renewed Ringer's solution the contractions become irregular again; similar to those before the muscarin was added. On now adding muscarin, in about five minutes a tracing similar to the above is obtained. The contractions, however, gradually become weaker, but retain their form to the end.

The stomach is gradually contracted, but very gradually. This thus differs from the relaxed condition of the heart under the action of the same drug.

With $\frac{1}{8000}$ the above was accentuated.

With $\frac{1}{4000}$ (Fig. 23) the first four spontaneous contractions were staircase in character, then they resumed their characteristic form.

With $\frac{1}{2000}$ the first twelve spontaneous contractions were also staircase in character, but of much greater height. The rest of the tracing was similar to the above, but the hardness and contracture on the following day were greater than the others.

Thus, even in strong solution, muscarin has little paralytic effect. In this respect it is similar to veratrin, and both are able to initiate rhythmical contractions, and cause a condition of rigor mortis. Of all the muscle stimulants, muscarin nitrate appears to be the best (on the stomach, however). On the heart, muscarin is said to stimulate the inhibitory ganglia. May not the great effect on the stomach be due to stimulating the plexus of Auerbach and muscle fibres as well?

Experiments on the antagonistic Action of some of the above Drugs—Atropin Sulphate and Muscarin Nitrate (Fig. 19).—When muscarin, $\frac{1}{10000}$, was added to atropin, $\frac{1}{10000}$, the irregular form of contractions got by the latter was altered to moderately regular but slightly larger ones. None of the characteristic contractions of muscarin were produced. On adding muscarin, $\frac{1}{2000}$ (Fig. 10), to a stomach which had been acted on by atropin, $\frac{1}{5000}$, the contractions peculiar to muscarin were produced; they were obliterated by atropin, $\frac{1}{5000}$, again. Thus muscarin can abolish the spontaneous contractions produced by atropin, and *vice versa*, while veratrin has no effect on the atropin curves. This result agrees with the experiments on the heart, atropin paralysing the inhibitory branches of the vagus, while muscarin stimulates them, each being capable of removing the effect of the other.

Veratrin Sulphate and Strychnin Sulphate.—Veratrin, $\frac{1}{5000}$, caused a rise of the lever, and the stomach went into a state of tetanic contractions. On adding strychnin, $\frac{1}{5000}$, the lever began to fall, and spontaneous contractions commenced.

Atropin Sulphate and Pilocarpin Hydrochlorate.—Atropin, $\frac{1}{5000}$ (Fig. 12), instantly abolishes the rhythmical contractions caused by pilocarpin, but they are again reproduced in a weaker form by pilocarpin, $\frac{1}{5000}$ after about twenty minutes' action.

In the above experiments, in no case is the result derived from one trial, many of them being repeated many times. Owing, however, to the slowness of contraction and constant attention which is necessary, much time and labour must be expended. In some of the Frogs also there appears to be a certain amount of idiosyncracy, as in the quinine experiment, from which Fig. 15 is taken. In this stomach, as noted in the text, relaxation instead of contracture occurred.

The above experiments were carried out in the Physiological Laboratory of the Owens College, under the direction and supervision of Professor Stirling, to whom I tender my sincere thanks for the kind assistance he has rendered me in many different ways.

EXPLANATION OF PLATE I.

In all figures, R = Ringer's solution. Vertical line = time of changing solution. $\frac{1}{10000}$, &c. = 1 part of drug in 10,000 parts of Ringer's solution. Drum revolves at rate of $\frac{7}{8}$ inch per 5 minutes. Tracings should be read from left to right →.

Fig. 1. Normal tracing form near the beginning of original tracing showing part of initial fall of lever.

Figs. 5 and 26. G = galvanic current.
,, 7 and 8. NaOH = caustic soda.
,, 9 and 13. $NaNO_2$ = sodium nitrite.
,, 10, &c. Atrop. = atropin sulphate.
,, 11, &c. Verat. = veratrin sulphate.
,, 12, &c. Piloc. = Pilocarpin hydrochlorate.
,, 14, &c. Q. = quinine sulphate.
,, 16, Strych. = strychnin sulphate.
,, 21, &c. Musc. = muscarin nitrate.

The remaining figures sufficiently explain themselves.

Dry Cover-Glass Microscopical Preparations.

BY

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By the researches of bacteriologists (especially Koch) on the one hand, and Ehrlich and his pupils on the other, we have been made acquainted with the advantages of "dry cover-glass preparations." In testing for the presence of bacteria in certain tissues or fluids—*e.g.*, sputum—it is usual to press a small piece of the tissue or a little of the sputum between two cover-glasses so as to spread it out as a thin film. On separating the two cover-glasses by gliding the one off the other, a thin film is left upon both cover-glasses. These films are allowed to dry, and can be stained afterwards by means of suitable dyes. This method was applied with great success by Ehrlich[1] and his pupils in order to study the "granules" or "granulations" of various kinds that occur in the colourless corpuscles of human and other blood.

I wish to draw attention to the advantages which this method affords for the study of many tissues, not only as a means of investigation of new phenomena, but also for securing suitable demonstrations of many objects for the student of histology.

Blood-Corpuscles.—Vincent Harris[2] has directed attention to this method as applied to the study of blood-corpuscles. The blood was spread in thin layers upon cover-glasses, and allowed to dry in direct sunlight. The dried blood was then covered with a solution of some aniline dye, and after a time the preparation was washed in water, dried in the flame of a spirit-lamp, and allowed to cool. A second dye was then used in the same way, and the preparation was mounted in balsam without being previously passed through alcohol and clove-oil, or other clarifying agent.

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[1] *Archiv. f. Anat. u. Phys.*, *Phys. Abth.*, 1879, p. 571; *Zeits. f. Klin. Med.*, Bd. I., p. 553; *Charité Annalen* 1884, p. 107; Eugen Westphal, *Ueber Mastzellen*, Inaug. Diss., Berlin, 1880; Nordmann, *Beiträge z. Färbung d. Mastzellen*, Inaug. Diss., Berlin, 1884.

[2] *Quart. Jour. Micr. Sci.*, XXIII. p. 792.

Harris also fixes the dried film of blood by means of a solution of chromic acid, $\frac{1}{12}$ per cent., or bichromate of potash, 0·5 per cent. It is then acted on by alcohol, washed in water, dried, and treated with a solution of Spiller's purple.

I have repeated this experiment, and find that most beautiful preparations of blood are thereby obtained by using various combinations of aniline dies for double-staining. In Frog's blood corpuscles, by using eosin and iodine green, the nucleus becomes green and the hæmogoblin a coppery tint. Harris has used many combinations of aniline dyes.

Following the directions of Ehrlich, it is easy for the student of histology to repeat Ehrlich's experiments, and convince himself of the existence of different kinds of "granules" in the leucocytes.

In the method of Ehrlich, in order to fix the blood-corpuscles on the cover-glass, the cover-glasses with the film of blood are subjected for several hours to a temperature of 120° C., and the temperature must be kept fairly constant. Instead of this drying process, the cover-glasses may be passed three or four or more times through the flame of a spirit-lamp or the flame of a Bunsen burner, as recommended by Koch for preparations of bacteria. The heating process is necessary for liquids that contain a large amount of albumin, so that this substance is changed from a soluble into an insoluble condition.

The following method by Nikiforow[1] may be substituted, viz., to rapidly coagulate the proteids by placing the cover-glasses in a fluid. The covers are dried by exposure to the air, and are afterwards placed for one or two hours in a mixture composed of absolute alcohol—which must be quite free from water—and ether, equal parts. The cover-glasses are then dried by exposure to the air, and are subsequently stained by the method of Ehrlich.

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[1] *Zeitsch. f. Wiss. Mikrosk.*, V, p. 340.

The most varied combinations of aniline dyes can be used in staining the blood corpuscles of the Frog or Newt or those of Man.

A very striking effect is obtained with a dry film of human blood treated in this way, and afterwards stained in a saturated watery solution of methylene blue. The cover-glass is floated in the methylene blue, film-surface next the fluid, and after several hours it is washed in water and alcohol, and either allowed to dry, and is then mounted in xylol-balsam; or, after it has been dehydrated in alcohol, it is clarified in xylol and mounted in xylol-balsam. The use of clove-oil is to be avoided, as it tends to destroy the colour of the aniline dyes.

In this preparation the coloured blood corpuscles are yellowish and unstained, while the leucocytes catch the eye at once by their pronounced blue colour. Some of them are fairly uniformly stained blue, while in others certain "granules" are stained blue. The student also sees at a glance the relative proportion of the colourless corpuscles.

By this means the long and tedious process of drying is avoided, so that the student can readily prepare for himself a great variety of combination of dyes for blood corpuscles.

Besides staining the blood corpuscles with one dye after another, a very good plan is to use Biondi's fluid, or rather Ehrlich's fluid as modified by Biondi.[1] Ehrlich's fluid consists of acid fuchsin and methyl blue or methyl green. The Ehrlich-Biondi fluid used is the same as that used by Heidenhain for the study of the structure of the villi of the small intestine. It consists of completely saturated watery solutions of the following:—100 c.c. orange, 20 c.c. acid fuchsin, and 50 c.c. methyl green. When required the solution is diluted in the proportion of 1 to 40 of water or thereby.

Leucocytes from Lymph-Glands.—Scrape the cut surface of a freshly-excised lymph-gland, and compress the scrapings between two cover-glasses, and stain them with methylene blue or Biondi-Ehrlich fluid.

The method, however, is equally applicable to other tissues besides blood. I find it specially well adapted for the study of epithelium.

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[1] Pflüger's *Archiv*, Supp., Bd. XLIII., 1888.

Epithelium.—Take, for example, ciliated epithelium and goblet cells. The cells are first dissociated in Ranvier's alcohol, *i.e.*, dilute alcohol (1 : 2), for twenty-four hours, and, for convenience, take the mucous membrane on the hard palate of the Frog. A thin film of the dissociated epithelium is pressed between two cover-glasses, and, after being dyed, they are immersed in the alcohol-ether mixture and then stained. A very good stain is methylene blue. The preparations are mounted in xylol-balsam. The cells retain their form; the nucleus is of a deeper blue than the rest of the cell, and, while the clear disc at the base of the cilia is particularly well-defined—sharper, in fact, than it is in a preparation mounted in glycerine or Farrant's solution.

The goblet cells are particularly beautiful. The contents of each cell are but slightly stained, while the nucleus is deep blue, and the small quantity of protoplasm surrounding it is of a lighter blue tint. Often the mucus projecting from the open mouths of the cells can be seen.

If a double stain be desired, steep the stained cover-glass, after staining with methylene blue, in absolute alcohol, clarify it with oil of cloves in which a little eosin is dissolved, displace the clove-oil by means of xylol, and mount in xylol-balsam. The nuclei and nucleoli now are blue, the rest of the cell of a light coppery or eosin tint.

Safranin also makes a good stain. The cells retain their shape wonderfully, and the preparations made in this way are certainly very beautiful.

Epithelium dissociated by dilute alcohol from any other epithelial surface may be used. Thus, the columnar and goblet cells of the Frog's stomach and of the intestine of the Frog or Newt, or the transitional epithelium of the bladder, make remarkably instructive and beautiful preparations. Stratified squamous epithelium from the œsophagus, *e.g.*, dissociated in a very dilute solution of potassic bichromate ($\frac{1}{8}$–$\frac{1}{50}$ per cent), for several days, when similarly treated, yields excellent preparations, which, after being heated, may be stained with methylene blue, methylene violet, or any other suitable dye. The same kind of preparation is readily made with the epithelium scraped from the dorsum of the tongue or inner surface of the cheek.

Bone Marrow.—Red bone marrow is so complex in its structure, and its constituents are so varied in their characters, that it is not easy to determine the relation of the one form of corpuscle to the other. The red marrow is readily obtained from the ribs of a Guinea-Pig or Rat, and if it be examined fresh in normal saline solution, one can see distinctly the various forms of leucocytes, imperfectly formed blood corpuscles, large cells with a twisted and convoluted nucleus, and the myeloplaxes or giant cells.

Place the red marrow in a small test-tube containing some normal saline fluid just tinged with methyl green. Shake up the fluid to diffuse and break up the red marrow. Allow the marrow to settle to the bottom of the test-tube, and spread a film of it between two cover-glasses. Stain and examine it. It may be stained in methylene blue or Biondi's fluid, and mounted in balsam. All the different kinds of cells can then be studied with great ease.

Nerve Cells.—Perhaps of all the tissues to which this method can be applied, there is none in which it yields such brilliant results as in connection with the multipolar nerve cells of the spinal cord. This method I first saw practised by Professor L. v. Thanhoffer in 1886, when I was in Buda-Pesth, and his remarkably able and beautifully illustrated monograph, published in Hungarian, has unfortunately not been translated into German. The results obtained by L. v. Thanhoffer by this method are given in a *résumé* published in German.[1]

The spinal cord should be taken from a freshly-killed animal, preferably a horse, but the spinal cord of a dog, an ox, or sheep does admirably. The larger the animal the better, for as a general rule the multipolar cells are larger in the larger animals. With a sharp-pointed pair of scissors pick out a small piece of the grey matter of the anterior cornu, and squeeze it between two cover-glasses. Separate them as already directed, and after they are dry stain them in methylene blue, either a watery or an alcoholic solution. I have chiefly used the former, and mounted them in balsam. The method is so simple and satisfactory that it can be used with success by the junior student of histology. It rarely fails, and one obtains a picture of the multipolar cells which it is difficult to describe. The processes, branched and unbranched, of the cells can be traced, many of them quite across the field of the microscope; the nucleus and nucleolus are sharply defined, while the body of the cell cannot be described either as homogeneous or granular, it is distinctly fibrillated, and the fibrils can be seen sweeping through the cell-substance in several different directions, and many of them can be traced either into the branched processes or axis-cylinder process of the nerve cells. I know of no method which yields such beautiful and satisfactory results, and is at the same time so simple in its execution as this one.

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[1] "Beiträge z. fein. Structur d. centr. Nervensystems," *Centralblatt f. Physiologie*, 1888.

Thanhoffer makes another form of preparation which is in some respects equally instructive, viz., to heat the thin film of nervous matter in the flame of a Bunsen burner until the film becomes brown. The nerve cells are "fixed," and are slightly "browned." They retain their shape and other characters, and present a strikingly beautiful picture in the field of the microscope. After cooling they are mounted in balsam.

It may be objected that many of the aniline colours are fugitive, and as a matter of fact many of them are. I have had in my possession for nearly three years nerve cells stained with methylene blue, and they seem as good as ever.

I have, however, found a simple method whereby these cells can be stained by means of carmine and mounted on cover-glass preparations. Macerate for one to four or five days, small parts of the grey matter of the spinal cord of a freshly-killed animal in the following mixture, proposed by Landois, for the dissociation of the elements of the nervous system:—Make saturated solutions of neutral ammonium chromate, potassic phosphate, and sodic sulphate, and take of each, 5 c.c., mix and add 100 c.c. water. After three or four days transfer the pieces of tissue to a fresh quantity of the mixture, and to it add an equal volume of picro-lithium carmine. After several days the nerve cells will be found deeply stained. Crush a small piece of the stained grey-matter between two cover-glasses. Mount in balsam. These preparations show distinctly the shape and other characters of the cells and their processes, but the protoplasm does not exhibit the striated appearance of the methylene blue specimens.

Blood Vessels.—In the preparations—both the methylene blue and the heated ones—of the spinal cord, the student will find beautiful specimens of the smaller blood-vessels, especially small arteries breaking up into capillaries. These methods give more satisfactory results, and with less trouble, than the one usually employed, viz., of washing and staining the fine flocculi projecting from the deep surface of the pia mater of the cerebrum.

Spermatozoa.—It is convenient to select the spermatozoa of the Newt and Rat, the former on account of their large size, and the latter because of their peculiar shaped head. Dry cover-glass preparations are made in the way already described, stained in methylene blue, and mounted in balsam.

This is one of the best methods of showing how different physically the head is from the tail or cilium of a spermatozoon. The heads of the spermatozoa alone are deeply stained with the methylene blue, so that on looking at the preparation one sees only the bright blue heads. If it is desired the tail may be stained with eosin coloured with clove-oil after dehydration in alcohol.

A very excellent method is to stain the Newt's spermatozoa in Biondi's fluid already referred to. By means of it the heads are stained of a reddish purple, the tail red, and the spiral filament also red, while the little junction piece, where the head joins the tail, is red also, but deeper in tint than either the tail or the spiral fibre. By this differentiation in staining the differences in the physical and chemical constitution of the several parts of a spermatozoon are readily demonstrated. The method is one which is obviously capable of great extension in its application, and it can be applied with success to the study of other organs and tissues besides those mentioned.

(Journal of Anatomy and Physiology, 1890.)

The Effect of Electrolysis and of Putrefaction on the Bile, and particularly on the Bile Pigments.

BY

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George Henry Lewes Student.

(PLATE II.)

WHEN ox-bile is allowed to stand, freely exposed to the air, the original green colour soon changes into brown and ultimately into yellow, and after a varying interval Gmelin's reaction is no longer given; that is to say, the bile pigment is converted into a yellow pigment which is not oxidised by nitric acid into bilirubin, biliverdin, and the higher products of oxidation of the normal bile pigment. This change is accompanied by putrefaction under the conditions described; but it also takes place in bile freed from mucin by precipitation with alcohol and then evaporated to dryness at a low temperature. Bile treated in this way remains free from putrefaction; nevertheless, the progressive changes in the bile pigment are not prevented, and a clear yellow mass is ultimately left, the pigment in which does not give Gmelin's reaction.

When bile is electrolysed in a U-tube, or in a vessel in which the electrodes are separated by a porous partition, changes take place in the pigment at the negative pole which are not unlike the changes produced when bile is allowed to stand. The bluish-green or green of the fresh bile passes through yellowish-green into golden-yellow, and, if the electrolysis is allowed to go further, this gives place to a pale-yellow shade. In the earlier stages of the electrolysis reversal of the current is sufficient to restore the original colour in the neighbourhood of the cathode, but after the electrolytic changes have decidedly developed themselves, it may be impossible to restore the pigment to its original state by the action of the positive pole. Even nitric acid may fail to oxidise it, and Gmelin's test may no longer succeed.

Thus we see that the same result may be produced by electrolysis in an hour or two, as is produced in months by the slower processes at work in bile which is simply allowed to stand—a progressive change in the bile pigment, which can be stopped and reversed in its earlier stages by oxidising agents (influence of the anode, nitric acid), but which becomes irreversible when it has gone a certain length. Although the gross result of the two processes is the same, we cannot infer that the processes themselves must be identical. Still the fact that the action of the cathode, which is certainly, to a large extent at least, a reducing action, produces similar effects on the bile pigment to those produced by simple standing, is an important hint that the latter are also to be ascribed to reduction. The anode has a very distinct oxidising action on the pigment. In the earlier stages of the electrolysis this action can be reversed by changing the direction of the current; but when a strong or long-continued current has carried the change of colour through green and bluish-green to the yellow of the highest stage of oxidation, the reducing action of the cathode may fail to reverse the cycle.

When ox-bile which has already undergone changes by standing, so that the colour has become yellow, but which nevertheless still gives Gmelin's reaction, is electrolysed, the action of the anode is able to oxidise the yellow pigment at least to the stage of biliverdin. This is true whether the change in the pigment has been accompanied by putrefaction or not. But where Gmelin's reaction is no longer given, whether putrefaction has been allowed to occur or not, the anode can no longer oxidise the yellow pigment.

After witnessing the very striking changes which the current produces in the colour of the bile pigments, I turned with considerable interest to the spectroscopic investigation of these changes, for I thought it might be possible, if any of the products of oxidation or reduction of the pigments possessed well-marked absorption bands, to make a finer analysis of such products in this way than in any other. It was somewhat disappointing then to find that amidst all this play of colour, while the bile pigments were running through the whole gamut of their chemical changes, the bands of the ox-bile spectrum stood their ground. No new bands appeared; no old bands faded out, with a moderately strong current. It was an excellent demonstration that the bands of ox-bile are not due to any of the bile pigments belonging to what we may call the normal series. Cholohaematin must be a substance differing much in stability, or at least in resistance to oxidation and reduction by the voltaic current, from the pigments of the bilirubin set. With strong currents, oxidation, the action of the anode, is that which seems to affect the bile spectrum most. It weakens all the bands, especially that in the red. Reduction, the action of the cathode, seems on the contrary to bring the bands out more sharply. But this is only in the first stage. When the electrolysis is powerful the cathode is no more favourable to the persistence of the bands than the anode.

And in agreement with this we find that the bands grow in strength and distinctness for a considerable time when bile is allowed to stand and putrefy, although ultimately, as putrefaction goes on, they disappear. (See Experiments 1 and 2.)

The *bile salts* are electrolytes, and in electrolysis of pure watery solutions of them an acid constituent is precipitated at the anode in the form of long needles. When bile is allowed to putrefy Pettenkofer's reaction is given for a long time, but ultimately fails. On the other hand, when bile is allowed to stand without putrefaction, after precipitation of the mucin by alcohol, and evaporation to dryness, Pettenkofer's reaction is given long after it has ceased to be given by the putrefying bile, and long after Gmelin's reaction has failed. Putrefaction does not seem to affect the fate of the bile pigment, as we have seen, except, perhaps, by hastening on the final stage. On the contrary, the breaking up of the bile salts is determined by putrefaction. The difference in nutritive value, for micro-organisms at least, of the salts and the pigment is evident enough.

Experiment 1.—Ox-bile was used. A current of 70 milliampères, 10 milliampères per square centimetre of section, was passed for an hour and a half through undiluted bile. Although it was only 20 hours since the death of the animal, and the weather very cold, the spectrum (Fig. 1, I.) shewed with great sharpness three out of the four bands which are generally described as being found only in ox bile which has stood for some time, and in which decomposition of the pigment has taken place. The bile seemed perfectly fresh. But it contained much bilirubin, and its colour was yellowish-brown rather than green. The reaction was slightly alkaline. At the end of the experiment a light-green slimy mass was found on the anode, and the reaction was strongly acid. Under the microscope the deposit was seen to be amorphous, consisting of masses of very fine granules. It was insoluble in water in chloroform and in ether, but freely soluble in alcohol and in dilute caustic soda, and gave Gmelin's reaction. It gave, in fact, all the reactions of biliverdin. Its alcoholic solution shewed the spectrum of Fig. 1, IV. When this deposit was filtered off from the liquid in the positive compartment, the filtrate was also light green in colour, gave Gmelin's reaction, and shewed the spectrum of Fig. 1, II. Dilute acetic acid caused no precipitate in the cold, the mucin having been thrown down by the electrolysis. In the cathodic compartment, the colour of the bile was nearly the same as it was originally, but there was less green in it. There was no deposit on the cathode. The reaction was strongly alkaline. Dilute acetic acid brought down a precipitate in the cold. The spectrum was that of Fig. 1, III. In both compartments the liquid was no longer viscid like ordinary bile. [The spectra were mapped with a Zeiss spectroscope with wave-length scale.]

EXPERIMENT 2.—Ox-bile was examined in less than an hour after the death of the animal. It showed already the spectrum of Fig. 1, VI., by far the best marked band being that over *C*. In 18 hours the spectrum was that of Fig 1, VII. The band between *D* and *E* was now very much more distinct than at first, and the others were also better marked. In 70 hours the spectrum was that of Fig. 1, VIII. The band over *C* is now much weakened, and there is a narrow band near *B*. The *D* band is also weakened, while that between *D* and *E* is very sharp. In five days from the death of the animal, the weather being warm, the spectrum was still that of VIII. In fifteen weeks the bands had entirely disappeared. Some of the fresh bile was electrolysed for two hours by a current of 70 milliampères mean strength. The spectroscopic investigation did not shew anything essentially different from the results of the last experiment. There was, however, more biliverdin present to begin with, and the change of colour in the cathodic compartment to yellowish green was better marked. There was a large deposit in this compartment consisting of beautiful sheaves of needle-shaped crystals, slightly coloured with the pigment, and mingled with numerous dumb-bell-shaped crystals and some amorphous material.

The sheaves, which seem to be a constant result of electrolysis, are insoluble in 95 per cent alcohol and in absolute alcohol both cold and hot; insoluble in dilute acetic and hydrochloric acids. In strong sulphuric acid they disappear and are replaced by isolated fine needles and granules. They are insoluble in sodium carbonate, caustic soda, and ammonia. The dumb-bells are soluble in dilute acids.

EXPERIMENT 3.—Ox-bile which had stood for six days, and was of a yellowish brown colour, was electrolysed by a current of 4 milliampères. In nine hours there was a turbid light green layer extending down one-third of the length of the anodic limb around and below the electrode. In the cathodic limb there was no obvious change except a slight turbidity in nearly the whole limb. The rest of the tube preserved the original colour. The current was now reversed. In 12 hours the upper half of the present anodic limb is light green and very turbid. The rest of the limb is clear and greenish yellow. In the present cathodic limb the upper two-thirds is yellow, with a tinge of red. Immediatly below this is a narrow sharply marked-off layer distinctly red. The rest of the tube is apparently unchanged. The negative electrode has an odour somewhat like that of trimethylamine, the positive has the aromatic odour of bile. V. in the figure shews the original spectrum. IX is that of the bile in the present cathodic limb immediately below the red layer. X is that of the uppermost layer of the present cathodic limb.

EXPERIMENT 4.—A solution of pure sodium glyocholate was electrolysed by a current of one milliampère. In half an hour long needle-shaped crystals began to separate out in the anodic limb of the U tube, first around the electrode and afterwards farther down, till in five hours the whole anodic limb was a crystalline mass. The cathodic limb remained clear. The crystals, so far as could be made out microscopically, resembled glycocholic acid, although there were some which seemed more like cholic acid. They were only slightly soluble in distilled water in the cold but dissolved partially on heating. Very readily soluble in dilute alkali. Gave Pettenkofer's reaction.

EXPERIMENT 5.—Pure sodium taurocholate solution was electrolysed. Crystals very like those of Experiment 4, separated out at the anode. A much larger proportion of the deposit, however, seemed to dissolve in cold water.

The bile salts then are electrolytes. Bile is therefore an animal liquid which does not conduct solely by its inorganic salts. Its conductivity is high. The bile pigments are very different. In pure solutions they conduct badly, and the changes produced in them by electrolysis of bile must be due to secondary actions.

Without venturing into the perilous path of the interpretation of absorption spectra, I may point out that a spectrum which is essentially that of the so-called cholohæmatin of MacMunn may be given by ox-bile examined almost immediately after the death of the animal, that this becomes more strongly marked when the bile stands, and that the action of the anode, which is so powerful upon the blood pigment, affects this spectrum chiefly by modifying the general absorption at the ends, shutting out the red and letting through more of the blue and green, but leaving what may be called the fundamental bands of the spectrum unaltered. One new band may indeed appear, under certain conditions, in electrolysis, immediately to the right of *B* (Spectrum IX.). But Spectrum VIII. shows that this may appear also in bile which is allowed to stand.[1]

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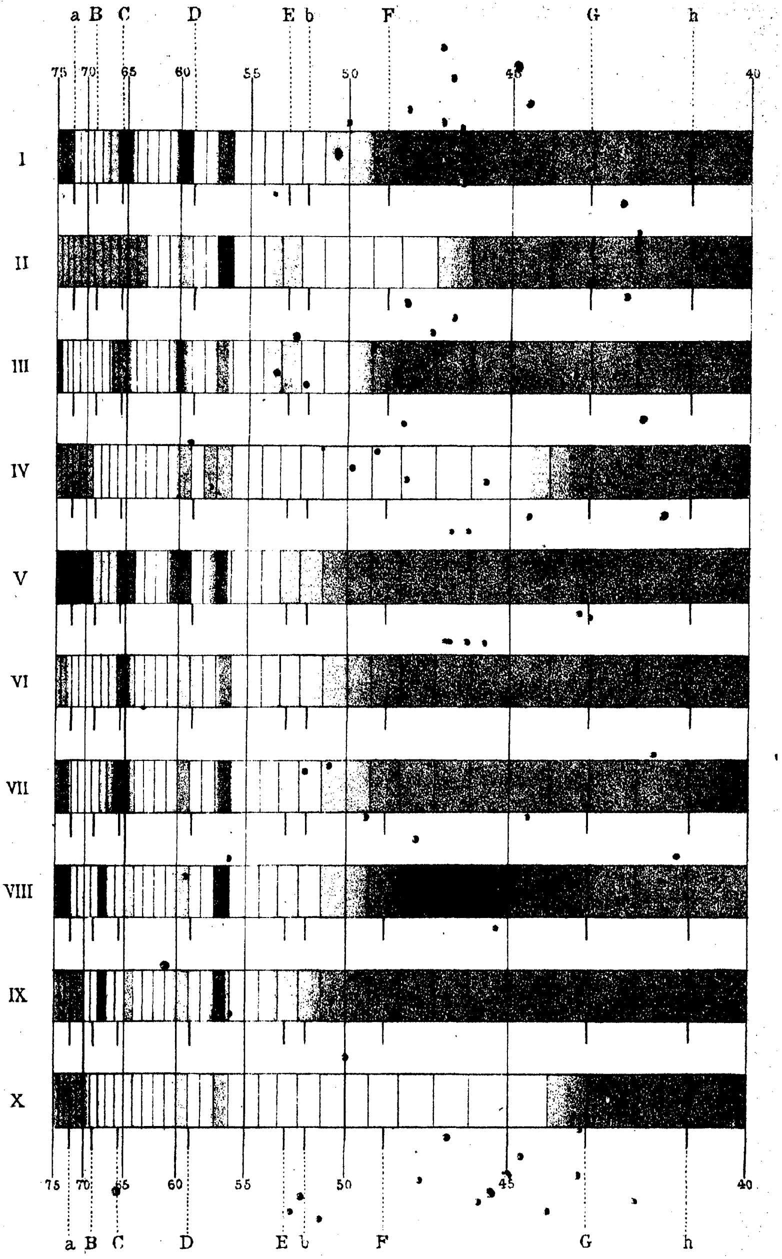
[1] Since the above was written I have seen a paper by Haycraft and Scofield in the *Zeitschrift für Physiologische Chemie*, Vol. XIV., 1890, p. 73, which deals with an analogous subject but on different lines. So far as the two papers overlap, I am happy to find a substantial agreement between our results.



F. Huth, Lith^r Edin^r

Further Observations on the Histology of Striped Muscle.

BY

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Late Platt Physiological Scholar in the Owens College.

(WITH ONE PLATE.)

IN a recent paper published in the *Quarterly Journal of Microscopical Science*[1] I gave the results of some researches on the histology of muscular fibre. The present paper is a record of my further investigations on this subject. These are somewhat incomplete owing to want of time to finish them satisfactorily, but several results have been obtained which I trust are worthy of publication.

In order to render the questions dealt with more intelligible the summary of results arrived at in the former paper is reproduced.

Summary of Former Paper.

1. In all muscles which have to perform rapid or frequent movements a certain portion of the muscle is differentiated to perform the function of contraction, and this portion takes on the form of a very regular and highly modified intracellular network.

2. This network, by its regular arrangement, gives rise to certain optical effects which cause the peculiar appearances of striped muscle.

3. The contraction of the striped muscle-fibre is probably caused by the active contraction of the longitudinal fibrils of the intra-cellular network; the transverse networks appear to be passively elastic, and by their elastic rebound cause the

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[1] "Observations on the Structure and Distribution of Striped and Unstriped Muscle in the Animal Kingdom, and a Theory of Muscular Contraction," *Quart. Journ. Micr. Sci.*, 1887.

muscle to rapidly resume its relaxed condition when the longitudinal fibrils have ceased to contract; they are possibly also paths for the nervous impulses.

4. In some cases where muscle has been hitherto described as striped, but gives no appearance of the network on treatment with the gold or other methods, the apparent striation is due to optical effects caused by a corrugated outline to the fibre.

5. In muscles which do not perform rapid movements, but where contraction is comparatively slow and peristaltic in nature, this peculiar network is not developed. In most if not all of the invertebrate unstriped muscle there does not appear to be an intra-cellular network present in any form; but in the vertebrate unstriped muscle a network is present in the form of longitudinal fibrils only; this possibly represents a form of network intermediate between the typical irregular intra-cellular network of other cells and the highly modified network of striped muscle.

6. The cardiac muscle-cells contain a network similar to that of ordinary striped muscle.

Discussion of the Views of Recent Observers.

Before commencing the subject-matter of this paper I propose to discuss the results arrived at by several recent observers concerning the structure of muscle-fibre, and also some of the criticisms which have lately appeared concerning the existence of an intra-cellular network in striped muscle.

The most important paper to discuss is that of Rollett.[1] He considers the muscle-fibre to consist of longitudinal fibrillæ grouped together into "muscle-columns," the cross-sections of which correspond to Cohnheim's areas. Filling up the spaces between the muscle-columns is the interfibrillar material or "sarcoplasma." Each muscle-column consists of alternating thick and thin segments; in the centre of each thin segment is a dark granule. The thick segments of the muscle-columns correspond to "Bowman's sarcous elements," and the dark granules in the centre of the thin segments correspond to

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[1] *Arch. f. Mikr. Anat.*, 1888, pp. 233–265.

"Krause's membrane" or the "transverse network" (Fig. 13 and diagram B).

The sarcoplasma is the part which is stained in gold preparations. In short, Rollett regards the appearance of gold preparations as due to the staining of the sarcoplasma, and considers this to be a honeycomb of interfibrillar material, and not a true intra-cellular network.

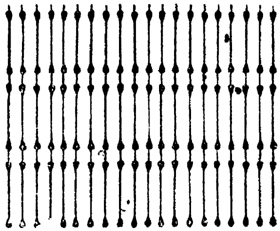

Diagram A (high focus).

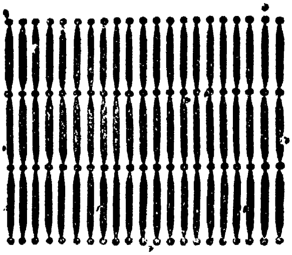

Diagram B (low focus).

The latter portion of Rollett's paper is devoted to an elaborate criticism of the network view of the structure held by Melland, Van Gehuchten, Carnoy, and myself. He states that what we describe as the fibrils of the network are only transverse and longitudinal sections of the walls of the honeycomb or sarcoplasma ("Was beide Autoren an diesen als Faden beschreiben sind nur Quer- oder Längsschnitte der Wände des Wabenwerkes, welches das Sarcoplasma um die Muskelsäulchen bildet," p. 252).

He describes the appearances seen in fresh muscle-fibre as follows:—At low focus (diagram (B) the muscle columns appear dark and in a line with the granules, the sarcoplasma appearing light. At high focus (diagram A) the sarcoplasma appears dark, the muscle-columns light, and two rows of granules appear in a line with the sarcoplasma and alternating with the muscle-columns. He states that the dark lines drawn by Melland joining the dark granules are only parts of the optical longitudinal sections of the walls of the sarcoplasma and are only seen at high focus; and that they do not lie in a line with the dark granules, but alternate with them, the dark granules being only seen at low focus. The granules which appear at high focus he considers to be thickenings of the sarcoplasma.

The above diagrams, modified from Rollett's figures, will make these points clearer. Diagram B represents the appearance at low focus, and diagram A the appearance at high focus according to Rollett. In B the muscle-columns appear dark and the sarcoplasma light; in A the sarcoplasma dark and the muscle columns light.

In the same way he states that the rows of granules seen in gold preparations are thickenings of the sarcoplasma lying between the thin segments of the muscle-columns, and that the true row of granules which correspond to and are parts of the muscle-columns are only seen at low focus, and then do not lie in a line with the dark lines but alternate with them.

He describes the same appearance at low and high focus in hardened muscle, and states that Melland and Van Gehuchten place the granules alternating with the muscle-columns, whereas they are really in a line with them.

In short, he concludes that a network does not exist, and that the appearances described by Melland, Van Gehuchten, and myself are due to errors in the interpretation of microscopic appearances and confusion of high and low focus ("Ich komme also zum Resultate dass ein Netzwerk im Sinne von Melland, Marshall, und Van Gehuchten und ein Enchyleme im Sinne des Letzteren in der quergestreiften Muskelfaser nich existirt," p. 262).

In answer to this criticism I venture to make the following observations.

1. If the appearance of the network in fresh muscle is due to the optical appearance of the "sarcoplasma" at high focus, there must always be a double row of granules—one on each side of Krause's membrane. (I use this term for the sake of convenience, and take it to represent the row of dark granules of Rollett's muscle-columns, and the row of granules of the transverse network of Melland, &c.).

2. If the granules seen at high focus in gold preparations are thickenings of the sarcoplasma stained by gold, there must

again be a double row, one on each side of the true row of granules which are only seen on the low focus, and further, the former rows of granules must alternate with the latter. (*Vide* diagram A.) Although I have examined several hundred preparations of muscle-fibre, I have never seen two rows of granules in any gold or fresh preparation, nor have I seen any change in the position of the granules at high and low focus. The granules have always appeared to me to be in a line with, and connected with the longitudinal lines of, the network (or "sarcoplasma" at both high and low focus.

3. Rollett states that the "sarcoplasma" is a honeycomb, and that the appearance of the network is due to the optical sections of the walls of that honeycomb. If this is true, I do not understand why there is never any appearance of honeycomb structure in finely teased preparations, whereas isolated pieces of network are easily obtained. Moreover, one preparation obtained by Melland is, I think, almost conclusive in favour of a network (Fig. 15).

4. According to Rollett, the "muscle-columns" are the essential parts of the fibre, and the "sarcoplasma" is simply interfibrillar material; we should therefore expect the latter to be least abundant in the most perfectly developed muscles. Now, in insects which possess the most powerful and most rapidly contracting muscles of all animals, the part stained by gold is more strongly marked than in other animals. This seems to point to the fact that it is the most essential part of the fibre and not interfibrillar material, and is therefore in favour of the network view.

5. Again, in developing muscle-fibre I have shown that the network is present but only demonstrated with difficulty; whereas if it were inert interfibrillar substance, one would expect it to be relatively more abundant in the embryonic fibre.

6. If, as I have attempted to show, the nerve-ending is connected with the part of the fibre stained by gold, this again points to the latter being the essential portion of the fibre; while if Rollett's view is correct, we should expect the nerve-ending to be connected with the muscle-columns.

7. The apparent connection in some cases of the network with the intra-nuclear network of the muscle-corpuscles is in favour of the network view.

8. Lastly, the network view places the muscle-cell on a basis of comparison with other cells having intra-cellular networks, whereas all other views of the structure are at variance with such a comparison.

Dr. Klein[1] adopts Rollett's view in the new edition of his text-book, and states that "the reticulation described by Melland, Marshall, and others, is due to the coagulation of the sarcoplasma brought about by certain hardening reagents."

Dr. Michael Foster apparently holds the same view of the structure in the new edition of his book,[2] for he states that the muscle-substance is composed of longitudinal fibrillæ, embedded in interfibrillar substance which stains with gold, and hence appears as a network. He says, "The interfibrillar substance is relatively to the fibrillæ more abundant in the muscles of some animals than in those of others, being, for instance, very conspicuous in the muscles of insects, in which animals we should naturally expect the less differentiated material to be more plentiful than in the muscles of the more highly developed mammal."

Now, I think I am right in saying that the muscular system of insects is the most highly developed in the animal kingdom; certainly the muscles are far more powerful in comparison with the size of the animal than in mammals, and among insects are found the most rapid movements. It therefore appears to me that the fact of the meshwork being more conspicuous in the muscles of insects is strongly in favour of its being the active part of the fibre, and not of the nature of interfibrillar substance.

I shall next give a *résumé* of the results of some recent observers who are in favour of the existence of a network.

Van Gehuchten[3] has described a network in striped muscle similar in most respects to that described by Melland, myself, and others, but differing in some details.

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[1] "Elements of Histology," p. 76.

[2] "Text-book of Physiology," vol. I, p. 91.

[3] "Etude sur la structure intime de la cellule musculaire striée." Extrait de la Revue *La Cellule*, t. II. 2 fascicul. Louvain, Gand et Liège, 1886.

Carnoy[1] also adopts the network view, and remarks that "La cellule musculaire est une cellule ordinaire dont le réticulum s'est régularisé, et l'enchylème chargé de myosine."

Haswell[2] has recently published an important paper on this subject. His observations were made on the gizzard of various species of Syllis, where he claims to have found very primitive forms of striped muscle. He divides striped muscle into simple and compound types, the simple type showing only transverse striation, not due to network; the compound both transverse and longitudinal. These two types correspond to what I have termed true and false striation; the characteristics of the compound or true striped muscle being as follows:

1. Each fibre consists of a bundle of fibrils.
2. Each fibre is composed of two alternating series of anisotropous and isotropous segments, the former of which are more easily stained than the latter.
3. Running across the fibre in the middle of each isotropous segment is the transverse network, or Krause's membrane.
4. Between the fibrils run the strands of the longitudinal network.
5. Each fibre is formed from a single cell, the nucleus of which divides and forms "a multinucleated protoplasmic body, by modification of whose protoplasm the muscle substance and networks are formed."

He states that in these animals the elements of the fibre are on a larger scale than in Vertebrates and Arthropods, and are hence favourable for the study of the structure of muscular fibre, which "seems to lead to that view of the structure of compound striated fibres advocated by Retzius, Bremer, Melland, C. F. Marshall, and others; the only point of importance in which there seems reason for dissenting from that view being with reference to the relation of the transverse networks to the fibrils." The chief differences between the network Haswell describes and that described by the above observers are these: (1) He states that the transverse networks are not only in the interspaces between the "fibrils," but partly also penetrate them. (2) The longitudinal networks, though mostly longitudinal, have oblique strands and anastomoses. (3) The transverse networks sometimes appear as rows of spindle-shaped granular bodies, but in crushed specimens he says these are seen to consist of a close reticulum of delicate threads, the spindle bodies being condensed parts of it. (4) The distance between the transverse networks is much greater.

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[1] 'La Biologie cellulaire,' 1884.

[2] *Quart. Journ. Micr. Sci.*, 1889.

Haswell regards the "fibrils" as the contractile part, and not the network.

I shall refer to Haswell's observations again in the subsequent portions of this paper.

A. B. Macallum[1] has published some interesting observations dealing with striped muscle. He confirms the existence of the network of Retzius, Melland, and others, and considers it the contractile part of the fibre. He states that the muscle-nuclei are marked by furrows, sometimes transverse and sometimes longitudinal, and that these are probably caused by pressure of the trabeculæ of the network. He also states that in some nuclei there is an intra-nuclear network similar to that of the fibre itself.

It thus appears that the view of the existence of a true intra-cellular network in striped muscle-fibre has received much support, and has been confirmed by several recent observers; and that the view held by Rollett and others that it is interfibrillar material has not sufficient evidence for it to be accepted. I shall therefore, in accordance with the results of Retzius, Bremer, Melland, Carnoy, Haswell, Macallum, and myself, assume that the former view represents the true structure of striped muscle.

The present paper dealt with the following points:

1. The connection of the transverse networks with the muscle corpuscles.

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[1] "On the Nuclei of the Striated Muscle-fibre in Menobranchus," *Quart. Journ. Micr. Sci.*, 1887.

2. The development of the network.
3. The connection of the nerve-ending with the network.

Connection of Network with Muscle-corpuscles.

It was shown by Retzius[1] that the transverse portions of the muscle network were directly connected with the muscle-corpuscles. He states that the protoplasm of the muscle-corpuscle is produced into several processes from which finer processes arise forming the transverse networks. Retzius' results were obtained by a modification of gold staining. The fresh muscle-fibre was first placed in a 1 per cent solution of formic acid for a few seconds, then in gold chloride $\frac{1}{4}$—$\frac{1}{2}$ per cent for twenty-five minutes, then in formic acid 1 per cent, and exposed to light for 10—20 hours.

By a special method of staining I have been able to confirm Retzius' results, and have made specimens showing the undoubted connection of the transverse networks with the muscle-corpuscles.

Method of Preparation.—The method of staining I adopted is a modification of that employed by Mays for demonstrating nerve-endings in muscle. He uses the following solution :

Arsenic acid $\frac{1}{2}$ per cent	·20 parts.
Gold chloride 1 per cent	4 „
Osmic acid 2 per cent	1 „

This solution, although it preserves the nerve-endings disintegrates the muscle-fibre. This I found was due to the arsenic acid. I therefore tried various strengths of acetic and formic acid in place of the arsenic, and found the best combination was the following :

Acetic acid 1 per cent	20 parts.
Gold chloride 1 per cent	4 „
Osmic acid 1 per cent	1 „

The muscle-fibre was placed in this solution for fifteen minutes, after previous immersion in acetic acid 1 per cent for a few seconds; then in acetic acid 1 per cent in a warm chamber for one or two hours.

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[1] "Zur Kenntniss der quergestreiften Muskelfaser." *Biologische Untersuchungen*, 1881.

1. *Dytiscus.*—Fresh muscle-fibre of Dytiscus stained by the above method shows the muscle-corpuscles in the form of one or more chains of nuclei in the substance of the fibre, the nuclei being surrounded by a small amount of undifferentiated protoplasm. The transverse networks are seen directly continuous with the nuclei. This is well shown in Fig. 1. Fig. 2 shows a portion of fibre with two rows of muscle nuclei; the transverse networks are seen to be connected with both sets of nuclei in some places. Fig. 3 shows several isolated nuclei with the transverse processes attached to them.

Transverse views of the network and nuclei are more difficult to obtain. Fig. 4 shows a transverse view of an isolated nucleus, with part of the transverse network connected with it.

2. *Dragon-fly.*—The muscle-corpuscles of the muscle-fibre of the dragon-fly are situated peripherally, *i.e.*, just under the sarcolemma, contrary to the general rule in insects.

In one preparation of this muscle I could trace the transverse networks into the muscle-corpuscles; and, moreover, the networks appeared to be distinctly connected with the intra-nuclear networks of the muscle-corpuscles (Fig. 5).

3. *Crayfish.*—In a preparation of crayfish muscle prepared by Retzius' method I could apparently trace the connection of the muscle network with that of the muscle-corpuscle. In this case it was somewhat difficult to tell whether the effect was not due to the network lying over the muscle-corpuscle; but by careful focussing I think the connection could be made out (Fig. 6).

These observations confirm Retzius' results, viz., that the transverse portions of the muscle network are directly connected with the muscle-corpuscles; and, furthermore, that the network is directly continuous with the intra-nuclear network of the corpuscles.

Development of the Network.

This I have studied in embryos of the trout and rat.

Trout.—In some gold preparations of embryo trout, taken from the ova, I found developing muscle-fibres in an early stage. These consisted of a portion of undifferentiated protoplasm containing the nucleus, and a portion already transversely striated. Under a comparatively high power the transversely striped portion showed darkly stained masses of an ellipsoidal shape arranged side by side, and causing the appearance of striation. Under a very high power ($\frac{1}{25}$ immersion) the network could be seen between these darkly stained masses, and in the same form in which it appears in the adult fibre. The dark masses appear to be some substance altered by the method of staining, and shrunken in the meshes of the network (Figs. 7, 8, and 9). No connection was seen between the network and the nucleus.

In older fibres the network is more fully developed, but still no connection appears to exist between the nuclei and the network (Fig. 10).

Rat.—In developing muscle-fibre from the embryo rat the fibres consist of an axial core of undifferentiated protoplasm containing the nuclei, and a peripheral part with developing network. Here, again, no connection was observed between the nuclei and the network.

It thus appears that—

1. The network appears at a very early stage.

2. It develops in its permanent form, and is not produced by the transformation of an irregular network into the adult type.

3. Each muscle-fibre is probably developed from a single cell, and is not formed by a coalescence of cells, either end to end or laterally (as Calberla[1] states), because the fibres of trout muscle examined were evidently single cells, and had the network well developed. It is difficult to conceive that these become fitted together, either end to end or laterally, so that the network of one cell should exactly fit on to that of the next.

4. The network develops centripetally, and commences at the part of the cell farthest away from the nucleus; moreover it

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[1] *Arch. f. mik. Anat.*, XI, 1875.

does not appear to become connected with the nuclei till the fibre is fully developed.

Haswell,[1] from his observations on the muscle of the gizzard of Syllis, forms a view of the ontogeny of striped muscles which does not agree with that described above.

In the same organ, in various species of Syllis, he finds in one case bundles of non-striped fibres; in another, compound hollow striated fibres, consisting of bundles of fibrils similar to the above, and bound together by a single transverse network. In a third there are three transverse networks, and so on up to the fully developed type of striped muscle found in Vertebrates and Arthropods. He therefore regards each striped fibre as derived from a bundle of non-striped fibres. He thinks the transverse network probably the equivalent of a transverse line of nuclei of the unstriped fibres, which occupies a similar position.

If this is correct each striped fibre must be a multicellular structure, and the network intercellular, and not intra-cellular. However, in another part of his paper he speaks of each fibre being formed from a single cell, the nucleus of which divides and forms "a multinucleated protoplasmic body, by modification of whose protoplasm the muscle-substance and networks are formed."

Connection of Nerve-ending with Muscle Network.

Nerve-endings may be divided into two main types, the circumscribed and the diffused form. Between these types there are many intermediate forms, as shown by Kühne in his classical work on "Nerve-Endings."[2] The nerve-endings in the muscle of the snake form a good example of the circumscribed variety. Here we have a localised end-plate, compact and non-ramifying. This is also the common type in mammalian muscle. In Dytiscus, on the other hand, we find the diffuse form, which branches and ramifies nearly to the end of the fibre.

In each case the nerve-ending, when stained by any of the gold methods, is seen to consist of a slightly stained ramifying portion, with darkly stained irregular masses between the branches of the lighter stained portions. The lighter stained part appears to be continuous with the axis-cylinder of the nerve.

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[1] *Loc. cit.*

[2] "Untersuchungen über motorische Nervenendigung." *Zeit. f. Biol.*, Bd. XXIII.

In attempting to demonstrate a connection between the nerve-ending and the muscle-network there is great difficulty, because the methods which best show the nerve-endings usually disintegrate the muscle-fibre and destroy the network. The best method of obtaining the nerve-endings is that employed by Mays[1] and also, with modifications, by Kühne,[2] viz., the mixture of arsenic acid, gold chloride, and osmic acid mentioned in the first part of this paper. But this method usually destroys the network, owing to the action of the arsenic acid. Another difficulty arises from the fact that the connection, if it exists, must take place underneath the nerve-ending, and hence in the normal position of the parts it could hardly be seen.

Dytiscus.—In one preparation of Dytiscus muscle prepared by May's method I succeeded in finding a portion of fibre with the network still intact (Fig. 11). A portion of the ramifying nerve-ending is seen crossing the fibre transversely; both the fibre and nerve-ending are stretched out transversely more than in the normal state. The nerve-ending appears to have been broken off from the upper part of the network and tilted over to show its inner surface, which here appears to be connected with the longitudinal bars of the network. The upper border of the nerve-ending, in the position of the figure, I take to be the external surface next the sarcolemma, the lower border to be the internal surface; the outline of the latter seems to me to point strongly to the fact that it is really connected with the longitudinal bars of the network.

Crayfish.—In preparations of crayfish muscle there are frequently found what I may, for want of a better term, call "streaks" of slightly stained matter usually crossing the fibre transversely. In some preparations these are to be seen distinctly continuous with the nerve-fibre going to the muscle, and are hence presumably portions of the nerve-ending. They appear to correspond to the lighter stained part of the nerve-endings as usually seen.

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[1] *Zeit. f. Biologie*, Bd. XX., 1884.

[2] Loc. cit.

In several specimens these streaks appeared to be connected with the longitudinal bars of the network, in the same way as in the specimen of Dytiscus muscle. Fig. 12 shews one of these streaks of nerve-ending connected with the network. The reason that these are so much stretched out appears to be on account of their connection with the network, which stretches them with it when it becomes stretched itself. In this figure the triangular deeply-stained bodies appear to represent the deeper stained part of the normal nerve-ending. They also appear to be continuous with the network at their apices.

Although the above results are imperfect, and not so conclusive as those discussed in the previous portions of this paper, nevertheless it appears to me that the nerve-ending is connected with the muscle-network, and apparently chiefly with the longitudinal fibrils of the network.

A recent paper by Macallum[1] on the termination of nerves in the liver of Menobranchus has an important relation to the above question. He states that he has traced the terminal fibrils of the nerves into direct connection with the intra-nuclear network of the hepatic cells, and the figures he gives seem to place this beyond doubt. This by analogy gives support to the view that the nerve-ending of muscle is connected with the network; and possibly this is the normal method of termination of nerves in connection with cells.

Haswell's[2] observations are also interesting in connection with this question. He describes a ganglion-cell at the end of each fibre, and occupying the axis of the fibre. This sends out numerous branching processes, which penetrate between the fibrils. He states that the core of the fibre is occupied by granular protoplasmic material, through which runs a network of fine threads, "connected with the fine branches of the nerve-processes of the ganglion-cell, and with the network of the muscle substance." Special branches of nerve-processes also ramify on the surface of the fibre, and probably enter into connection with the transverse networks.

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[1] *Quart. Journ. Micr. Sci.*, March, 1887.

[2] *Loc. cit.*

Hence both Macallum's and Haswell's observations lend support to the view that the network is connected with the nerve-ending.

Conclusion.

In reviewing the foregoing observations we are at once met by an apparent contradiction, viz., that in the young muscle-fibre the network does not appear to be connected with the nucleus, whereas the connection is definitely established (Retzius, Marshall) in the adult fibre. This at first sight is an absolute contradiction: for, on the one hand, it is difficult to conceive that processes originating as outgrowths from the nucleus could exactly hit off and fuse with the already formed network; on the other hand, it is almost equally inconceivable that the fibres should grow into the nucleus. This apparent contradiction can, I think, be to a great extent explained. Firstly, we must bear in mind that histological differentiation proceeds during its development from without inwards, *i e*, centripetally; and that the special characters of the adult cell appear first and are most marked at the periphery, *i.e.* farthest from the nucleus. Examples of this rule are seen in the development of epidermic cells, dentine, cartilage, and bone. In bone and dentine the processes are not supposed to grow from the cells, but to be formed by the lengthening of connecting strands by deposit of new matter.

Secondly the nerve-ending being on the surface of the fibre, and the network also appearing first on the surface, the connection between the two can be established from the first; whereas if the network grew out from the nuclei it could only be connected with the nerve-ending at a much later period. Hence the fibre would be useless till it was far advanced in development, because for the network to be of any use it must from the first be connected with the nerve-ending. For a new structure of any kind to be developed it must always have been of use from the first, either for its ultimate purpose or for some other. If the network grew out from the nuclei it could not be of use till it got to the nerve-ending, *i.e.*, to the surface.

In considering this point we may imagine that the cell divides into "formed and unformed matter" (Beale), the formed matter being characteristic of the particular cell. In the case of the muscle-fibre the protoplasm divides into network and muscle-plasma all along, beginning at the periphery and gradually extending to the nucleus. We are here met by an apparent difficulty, for in muscle-fibre the "formed matter" which is characteristic of the cell is the muscle-plasma, and not the network (which is presumed to be the contractile part). But the special feature of striped muscle is not the fact that it contracts, but the mode in which this is brought about, *i.e.*, the rapidity; and this will be a matter of nutrition which will depend on the muscle-plasma. Therefore the special feature of striped muscle is probably the mode of nutrition of its specially contractile part, *i.e.*, quick repair. The active network bathed in such a fluid is placed in a good position for such rapidity.

Summary.

1. The transverse portions of the network of the striped muscle-fibre are directly connected with the muscle-corpuscles.
2. The nerve-ending appears to be connected with the muscle-network, and chiefly with the longitudinal bars of the network.
3. The development of the network takes place at a very early stage in the development of the fibre, and the network develops from the first in its permanent form.
4. The network develops first at the surface, and grows centripetally. It does not appear to be connected with the muscle-corpuscles till the fibre is fully developed.
5. Each muscle-fibre appears to be developed from a single cell, and not by a coalescence of cells.

The investigations described in this paper were carried on in the Physiological Laboratory of Owens College during the winter of 1887. My thanks are due to the Council of the College for a special grant to enable me to carry on the research.

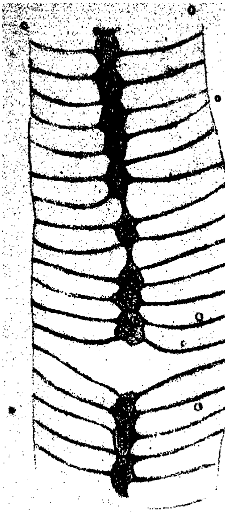

Fig. 1.
× 490

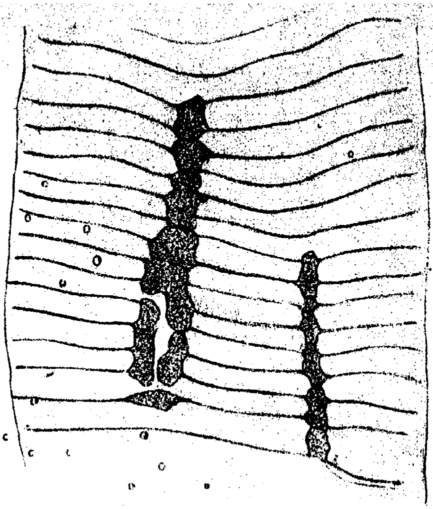

Fig. 2.
× 1090

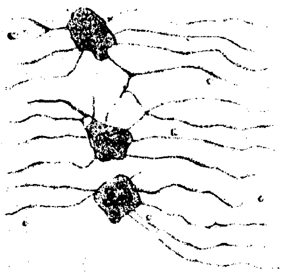

Fig. 3.
× 1090

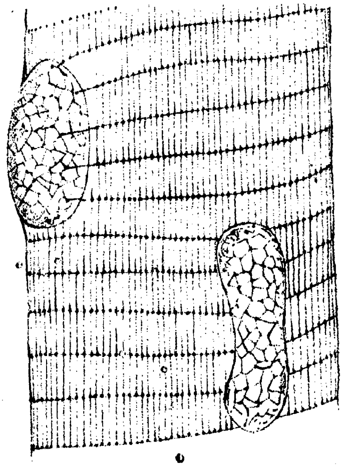

Fig. 5.
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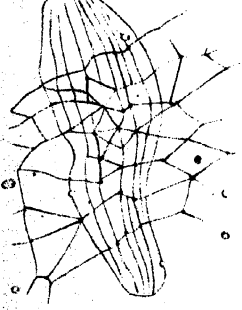


Fig. 6.
× 1090

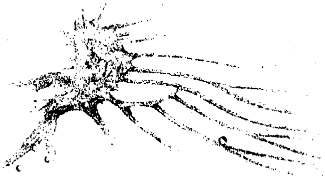


Fig. 4.
× 890

.7.
90

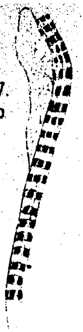


Fig. 8.
x 890

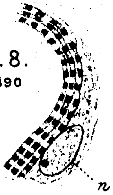


Fig. 9.
x 2800

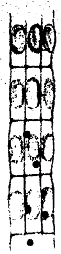


Fig. 10.
x 890

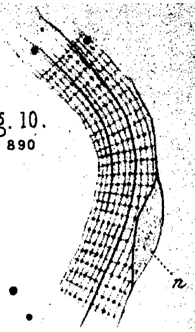


Fig 11.
x 1090

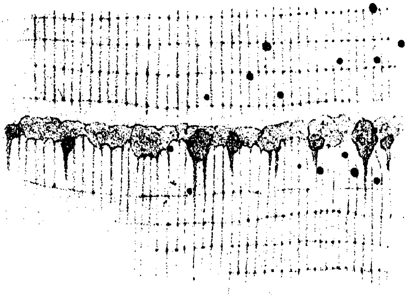


Fig 13.

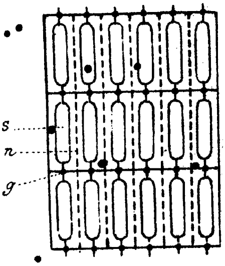


Fig 14.

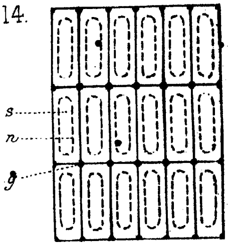


Fig. 12.
x 890

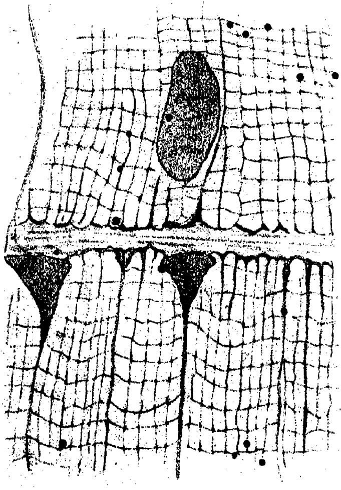
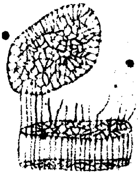


Fig 15.



F. Huth Lith^r Edin^r

I must express my thanks to my brother Professor Milnes Marshall for many valuable suggestions in producing this paper, and for examining several of the preparations. I must also thank Professor Stirling for much assistance to me in my work.

DESCRIPTION OF PLATE,

Illustrating Mr. C. F. Marshall's paper, "Further Observations on the Histology of Striped Muscle."

[The main details were drawn under the camera in all the Figures.]

FIG. 1.—Muscle-fibre of Dytiscus, showing transverse networks connected with muscle-corpuscles. The longitudinal bars of the network are omitted for the sake of distinctness. $\frac{1}{5}$th obj. Acetic acid, osmic acid, and gold chloride.

FIG. 2.—Portion of another fibre, with transverse networks connected with two rows of muscle-corpuscles. $\frac{1}{10}$th immersion obj. Same method.

FIG. 3.—Three isolated muscle-corpuscles of Dytiscus muscle, with transverse networks attached. $\frac{1}{10}$th immersion obj. Same method.

FIG. 4.—Muscle-corpuscle of Dytiscus, with portion of transverse network connected with it. Transverse view. Gold preparation. $\frac{1}{10}$th immersion obj.

FIG. 5.—Muscle-fibre of dragon-fly, showing two muscle-corpuscles with their intra-nuclear network. Some of the transverse networks are seen to be connected with the intra-nuclear network of the upper corpuscle. $\frac{1}{10}$th immersion obj. Acetic acid, osmic acid, and gold chloride.

FIG. 6.—Muscle-corpuscle of crayfish, with portion of the muscle network apparently connected with its intra-nuclear network. $\frac{1}{10}$th immersion obj.

FIGS. 7 and 8.—Developing muscle-fibres of trout, showing striation. *n.* Nucleus. $\frac{1}{10}$th immersion obj. Acetic acid 2 per cent, a few seconds; gold chloride one per cent, fifteen minutes; formic acid 25 per cent, thirty minutes in warm chamber.

FIG. 9.—Portion of the striated part of one of the above fibres, showing the network and the darkly stained bodies in its meshes. $\frac{1}{25}$th immersion obj.

FIG. 10.—More fully developed fibre of trout. *n*. Nucleus. $\frac{1}{10}$th immersion obj.

FIG. 11.—Muscle-fibre of Dytiscus, with a portion of nerve-ending apparently connected with the longitudinal bars of the network. $\frac{1}{10}$th immersion obj. May's method.

FIG. 12.—Muscle of crayfish, showing a "streak" of nerve-ending apparently connected with the longitudinal bars of the network. $\frac{1}{10}$th immersion obj. Retzius' method.

FIGS. 13 and 14.—Diagrams comparing the view of Rollett and others with the network. In Fig. 13 the structure, according to Rollett, is marked in full lines, and the networked marked in dotted lines. In Fig. 14 the thick segments of Rollett's muscle columns are shown by dotted lines in the meshes of the network. *n*. Network. *s*. Muscle-columns. *g*. Granules.

FIG. 15.—Portion of muscle-fibre of Dytiscus, showing network very plainly. One of the transverse networks split off, and some of the longitudinal bars are shown broken off. (Copied from Melland, *loc. cit.*, Fig. 6).

[*Note.*—In Figure 13 the lines connecting the thick parts of Rollett's muscle-columns should be much thicker. They represent the thin segments of the columns].

Some Recent and some New Histological Methods.

BY

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Brackenbury Professor of Physiology and Histology in The Owens College.

Dry Method for Blood-Platelets.—The methyl-violet and osmic acid methods for obtaining these bodies are well known. One of the most convenient methods, however, is that of Hayem.[1] Clean a slide, sterilise it in the flame of a Bunsen burner, and allow it to cool. Obtain a drop of blood from the finger in the usual way, and rapidly transfer a little to a slide. With the edge of another slide rapidly spread the blood into a very thin film on the sterilised slide. Move the slide to and fro in the air until the film dries. The whole process should not occupy more than four seconds. Cover the film with a cover-glass, and cover the edges of the latter with paraffin wax. The blood-platelets are readily seen in the dry film of blood amongst the other corpuscular elements.

Staining of Fibrin.—In a preparation of blood Spiller's purple is frequently used to stain fibrin. A much better method is that of Weigert, although the technique of the process is not so simple. It requires a little care, and after one or two attempts one readily succeeds. Suppose one has to deal with a thrombus in a vein, or a lung affected with acute croupous pneumonia. The tissue is hardened by the ordinary methods, and sections are made say by freezing. Stain a section for a minute or two in the following fluid :—

Gentian-violet (5 per cent)	44 c.c.
Aniline oil	1 c.c.
Alcohol (96 per cent)	6 c.c.

Place the section on a slide and remove as much of the stain as possible, by pressing on the section—and do this rather firmly—several folds of unsized printing paper. Ordinary filter paper or blotting paper must not be used, as the fibres of the paper adhere to the preparation. Pour on the section a small quantity of a solution of iodine (iodine dissolved in water containing five per cent of potassic iodide). The iodine solution rapidly decolorises most of the stained parts. A similar solution is used in Gram's method of staining certain bacteria. Remove the iodine solution in the same way as directed for the removal of the gentian-violet.

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[1] *Du sang et de ses altérations anatomiques.* Paris, 1889.

Pour on the section a mixture of equal parts of aniline oil and xylol. This is a clarifying medium, and will clarify sections even when the latter have not been previously dehydrated by alcohol. Move the fluid over the preparation by tilting the slide from side to side, much as a photographer moves a fixing fluid on a photographic plate. Remove the clarifying medium, and add a fresh supply of the same. It clarifies the section. and at the same time removes some of the dye. As long as there is a trace of white in the preparation, the latter is not completely dehydrated. As the aniline oil would ultimately destroy the colour of the section, remove with unsized paper as before as much as possible of the clarifying reagent, and wash the section several times with xylol. Mount the section in xylol-balsam; all the fibrils of fibrin have acquired a violet colour, and the other tissues are either unstained or only feebly stained.

Isolated Epithelial Cells.—The methods employed are exceedingly numerous, including dilute alcohol and very dilute solutions of chromic acid, or certain of the salts of the latter. Every histologist is familiar with the valuable properties of Ranvier's dilute alcohol. For epithelial structures, however, which are rather thick and dense, and where isolated cells are required for a large class of students, the following plan of Schiefferdecker[1] is most convenient. It is applicable to all forms of epithelium, but is specially valuable for stratified squamous epithelium, *e.g.*, of skin or mucous membranes. After experimenting with a great variety of tissues covered with stratified squamous epithelium, I find that the hard pad on the jaw of the sheep or ox answers admirably. Epithelial cells of all shapes, and prickle cells as well, can be readily isolated by the method. I am in the habit of isolating the cells, and keeping them when isolated in glycerine jelly. The cells may be stained beforehand, and kept ready stained in glycerine jelly. A drop of the glycerine jelly, when melted, contains a large number of isolated cells.

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[1] *Zeitschrift f. wissen. Mikroskop.*, Vol. III., p. 483, and *Das Mikroskop. u. d. Methoden.*, &c., by Behrens, Kossel, and Schiefferdecker, Braunschweig, 1889.

Make a cold watery extract of the "pancreaticum siccum" of Dr. Witte, of Rostock. This is supplied in commerce as a greyish-brown powder, which has marked digestive properties. It is prepared from the pancreas, and contains the same enzymes as the pancreas itself. Filter the extract, and into it place small pieces of the object to be digested. Cover the vessel, keep it at a temperature of 37° to 38° C. for three or four hours. Wash the tissue in water, and preserve it in a mixture of equal parts of glycerine, water, and alcohol (96 per cent.). After it has been macerated for a short time in this fluid, on scraping the surface isolated cells are detached, and can readily be examined, as they are, or after being stained by any of the staining reagents in ordinary use.

Mitosis in the Salamander.—Considering the importance of mitosis, and the fact that the process is described in every text-book of histology, one would think that a practical acquaintance with the various appearances should be within the reach of students. It is well known that the young stamens and unripe ovaries of certain plants yield excellent mitotic figures, and such tissues may be used. In some respects the study of the process is more difficult in animal textures. Keep the young of the Salamander until they are 1 to 1½ inch in length, but they must be well fed, else the mitotic figures are not well developed. Harden them in one-sixth per cent. chromic acid, or Flemming's mixture. Make transverse sections across the tail, so as to include the epithelial covering. Fix the sections on a slide with any of the recognised "fixatives," *e.g.*, oil of cloves and collodion, or white of egg and glycerine. Stain the sections in saffranin, treat them with acid-alcohol, and mount them in balsam. In almost every section the student will obtain nuclei in various phases of mitotic division, so that in a few sections he will possess nearly a complete cycle of mitotic changes.

For surface views the epithelium covering the gills or cornea of the young Salamander are also to be recommended.

Mitosis, however, is not so readily demonstrated in mammalian tissues, partly because the histological elements are smaller, and therefore it requires a much higher power to see the changes in the nuclei. In the amnion of the Rat, however, as pointed out by Solger,[1] mitotic figures are fairly readily obtained thus:—The uterus is cut out of a pregnant white rat just killed, and placed in a saturated watery solution of picric acid. The uterus is opened under the picric fluid, and the amnion liberated. The whole amnion is hardened for twenty-four hours in picric acid, and after thorough washing with alcohol, it is hardened in the various strengths of alcohol (beginning with 70 per cent.), and the membrane is then stained with Ehrlich's acid, hæmatoxylin, diluted one-half with water, and mounted in balsam. In this preparation the mitotic figures are fairly readily seen, but they are neither so large as in the Salamander, nor so small as those in the spleen or lymphatic glands. Still the mitotic figures are of fair size, the interval between the two poles of diaster being 0·01322 mm. The amnion is sufficiently large to enable one to get a considerable number of preparations from a single fœtus. The length of the fœtus from which the amnion is taken should be about 1·8 cm. to 2 cm. The mitosis shown here is in the epithelial cells covering the amnion. I have had the privilege of inspecting Professor Solger's preparations, and on repeating the process I find it to be one quite within the range of subjects that may be given to advanced students of histology.

Method of Martinotti and Resegotti for Mitosis.—The tissue, *e.g.*, a rapidly growing tumour, a freshly excised wart or papilloma, are hardened in absolute alcohol. Stain the sections in a watery solution of saffranin. The decolorisation in this method is accomplished by means of a hydro-alcoholic solution of chromic acid, made by adding eight parts of alcohol to one part of a one-tenth per cent. solution of chromic acid. By exposing the section for a short time to the action of this fluid, all the parts are decolorised except the chromatin fibrils of the nuclei. The sections are clarified in oil of bergamot and mounted in xylol-balsam. The complete process is the following:—The tissues are fixed in absolute alcohol. The sections are stained for five minutes in a saturated watery solution of saffranin, and are then transferred to the hydro-alcoholic solution of chromic acid to differentiate the colour. The sections remain in this fluid for about one minute or less, and are then dehydrated and clarified, as already described.[1]

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[1] "Säugethier-Mitosen im histolog. Kursus.," Schultze's *Archiv*, XXXIII. p. 517.

By this method the chromatic filaments and the nucleoli are coloured of a bright rosy tint, while the protoplasm and intercellular substance remain uncoloured. Resting nuclei are feebly stained of a pale rosy tint. The hydro-alcoholic chromic solution should be prepared each time it is required.

Resegotti has studied the action of a large number of aniline dyes on mitotic figures. The best results, with tissues hardened in absolute alcohol, are obtained by double staining with methyl-violet or dahlia with eosin or acid fuchsin. The sections are first stained in a weak solution of methyl-violet, and then in a very dilute alcoholic solution of eosin. In this process the chromic acid differentiating medium is omitted.[2]

Methods of Staining Elastic Fibres.—Considering the great importance of elastic fibres in the structure of so many organs, it is not to be wondered at that many methods have been proposed for facilitating the study of this tissue. Some years ago, relying on the indigestibility of elastic fibres by gastric juice, I devised a method of artificial digestion, whereby the whole fibrous tissue was dissolved by a peptic digestive fluid, leaving the elastic fibres unaffected.[1] Others have gone on a different principle, notably Unna,[2] Lustgarten, who used Victoria blue (24 hours),[3] Martinotti, and Herxheimer. I have tried all the recently described methods, but the two which seem to yield the best results are those of Herxheimer and Martinotti.

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[1] "Ulteriori esperienze sulla coloraz. d. figure cariocinet," *Giornale della R. Accad. di Med.*, 1888, Nos. 6, 7.

[2] "Un metodo per rendere evedenti le fig. cariocinet," by G. Martinotti and L. Resegotti, *Zeits. f. wiss. Mikros.*, IV. p. 326.

Herxheimer's Method for Elastic Fibres.[4]—Sections of a tissue or organ containing elastic fibres, and previously hardened in Müllers's fluid, are placed for a few minutes in an alcoholic solution of hæmatoxylin, to which is added a few drops of a saturated solution of lithium carbonate. The sections become quite black, and are transferred for five to twenty seconds to dilute tincture of perchloride of iron. This rapidly decolorises all the tissues except the elastic fibres, which remain of a blackish or bluish-brown tint. The sections are mounted in xylol-balsam. This method is very good for arteries and for the elastic fibres in the trachea and bronchi, and also for the elastic fibres of the cutis vera.

G. Martinotti's Method for Elastic Fibres.[5]—This seems to me to be one of the best methods for staining elastic fibres. The tissue or organ is hardened in ·2–·25 per cent. chromic acid, and afterwards in alcohol. Sections, after thorough washing, are steeped in an alcoholic solution of saffranin—0 for twenty-four or forty-eight hours or longer, in fact, they take no harm from prolonged immersion in saffranin, as any surplus dye can be washed out of the section by means of acid-alcohol, *i.e.*, alcohol containing 1 per cent. of hydrochloric acid. The solution of saffranin used is 5 grammes of saffranin dissolved in 100 c.c. alcohol, to which is added next day an equal volume of water, or thrice the volume of water may be added. I was somewhat surprised at first on trying this reaction to find that I did not succeed. Ultimately I found, however, that the cause of my want of success was due to the saffranin which I used, and which I procured in this country. On procuring a sample of saffranin—O, I at once obtained the result described by Martinotti, viz., coloration of the elastic fibres of a bluish or blackish-purple tint—or the fibres may be quite black—while certain of the other elements, *e.g.*, the nuclei, are stained red, and other elements diffuse red tint. The sections, over-stained in saffranin, are partially decolorised in acid-alcohol, and then mounted in xylol-balsam.

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[1] "A New Method of Preparing the Skin for Histological Examination," *Journ. Anat. and Phys.*, 1875.

[2] *Monatsch. f. prakt. Dermat.*, B. II., 1883 and 1886, p. 242.

[3] *Wien. Med. Jahrh*, .1886.

[4] *Fortschr, d. Med.*, Bd. IV. p. 785.

[5] "Un metodo semplice per la colorazione delle fibre elastische," *Zeitsch f. wissen. Mikros.*, IV. p. 31.

This is a most excellent method. I have used it specially for the elastic fibres in arteries. By this method the student gets a good idea of the distribution of the elastic structures—membranes and fibres—in the various coats of an artery. It is also most serviceable for sections of the lung. In this case the elastic network between the air-vesicles can be traced quite readily, and so can the elastic fibres in the deep layer of the pleura. One can readily trace the connection of the latter system of elastic fibres—epi-pulmonary—with the intra-pulmonary system of elastic fibres. Similarly, sections—transverse and longitudinal—of the trachea and bronchi yield good pictures. It is also very serviceable in the case of the spleen, revealing the extent and ramifications of the elastic fibres in the trabeculæ and in the intra-splenic arteries and veins. By it also the distribution of elastic fibres in the cutis vera is made fairly distinct, although we still require a good description of the arrangement of the elastic fibres in the cutis vera and its papillæ.

The coloration may be effected more rapidly by keeping the sections in the saffranin solution at a temperature of 37–38° C. for five hours or so.

Ferria[1] finds that even tissues hardened in absolute alcohol give the reaction, provided the sections are kept for at least five hours in a watery solution of chromic acid (1 : 1000) at a temperature of 37° C. or thereabout. They are then washed and stained in saffranin.

Elastic Fibres in the Lung.—I have devised the two following methods, which give good results:—Make thin sections of a dried and distended lung, and stain the sections on a slide with very dilute magenta. Care must be taken that the dry sections do not curl up, which they readily do when fluid is applied to them. Remove the surplus magenta and allow the section to dry completely on the slide. When the section is completely dry, add a drop of balsam and cover the preparation. The elastic fibres are stained red and their distribution and arrangement in and between the walls of the air-vesicles can be seen distinctly.

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[1] "La colorazione d. fibre elastiche coll' acido cromico e coll. saffranin," *Giorn. della R. Accad. d. Med.*, 1888, No. 6–7.

Instead of magenta methyl-violet may be used. The sections are then clarified by a mixture of aniline oil and xylol, and mounted in balsam.

Sarcolemma of Striped Muscle.—The usual plan is to direct that a piece of muscle be teased in distilled water, so that a bleb may be formed at one or more parts on the surface of the fibre. This method is by no means so very successful, and I daresay most teachers of Practical Histology have experienced some difficulty in enabling every student to secure a good preparation. A far better plan is that of Solger.[1] Place small pieces of fresh muscle in a saturated solution of ammonium carbonate (*saturated in the cold*) for a few minutes (3–5). Tease it slightly. It may remain in the fluid with advantage for a longer time. The sarcolemma is rapidly raised in the form of a clear bleb for a considerable distance along the course of the fibres, and there is not unfrequently here and there a small quantity of sarcoglia adhering to its under surface. The reaction seems to succeed better on animals (frogs) that have been kept in confinement for some time.

To see the *ruptured sarcous substance* and the sarcolemma stretching between the ends of the ruptured fibre, one of the best methods is the following:—Tease out a small portion of a fresh striped muscle in normal saline and place a hair—preferably from the beard—across the direction of the fibres on the slide. Apply a cover-glass, and press the latter down firmly on the preparation. The flattened hair ruptures the fibres, the sarcous substance retracts, and the course of the hair—after the latter is removed—is marked by a line showing the sarcolemma of the individual ruptured fibres.

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[1] "Kohlensaures Ammoniak, ein Mittel zur Demonst. d. Sarkolemmas," *Zeits. f. wissen. Mikros.*, VI. p. 189.

Muscle Discs.—The usual plan is to place dead muscle for several days in ·2 per cent. hydrochloric acid. This is often not a satisfactory method. I find that maceration for several hours in a saturated solution of ammonium carbonate rapidly effects the transverse cleavage of a striped muscular fibre into muscle discs.

Heidenhain's Method for Glands.—This method is applicable to all secretory glandular structures and to connective tissue as well. One obtains very instructive preparations of the salivary glands and pancreas by this method. The gland is hardened in alcohol, and small pieces of the hardened gland are placed for six to eight hours in 10 c.c. of 0·5–1 per cent. watery solution of hæmatoxylin; and then for an equal period in a few c.c. of ·5 per cent. solution of potassic bichromate or 1 per cent. watery solution of alum. The pieces look quite black when they are removed from the hæmatoxylin fluid. In this method the change of colour in the hæmatoxylin caused by the chromium salt or the alum is effected within the tissue itself. In the submaxillary gland, for example, the nuclei are stained of pleasant bluish-black tint, the cell-substance is steel grey, while the demilunes stand out distinctly.

This method does also very well for serous salivary glands such as the parotid, and also for the pancreas.

Heidenhain,[1] I think, has recently altered somewhat the method for the better. The tissue, hardened in alcohol, or a saturated solution of picric acid, is placed for twelve to twenty-four hours in $\frac{1}{3}$ per cent. of a watery solution of hæmatoxylin, and subsequently in ·5 per cent. solution of potassic chromate, in which it is bleached somewhat. By employing small pieces of tissue a differentiation of the chromatin can be obtained.

Recently a large number of new methods have been recommended for the study of nerve fibres. While the ordinary method with silver nitrate does very well for Ranviers's crosses, I think the following method is preferable; besides it has the advantage of revealing very sharply the alternate brown and clear transverse lines in the axial cylinder known as Frommann's lines:—

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[1] *Arch. f. mik. Anat.*, Bd. XXVII. p. 383.

Ranvier's Crosses and Frommann's Lines.—Place a fresh nerve in ·5–1 per cent. silver nitrate solution for twenty-four hours, and keep it in the dark. After the expiry of this time remove the nerve, wash it in water, and expose it to light for two to three days in equal parts of formic acid, glycerine, and water, and preserve it in glycerine. The crosses are sharply defined, and so are Frommann's lines; and if an axial cylinder happen to be detached from its position within a nerve fibre, the biconical swelling at the node can be readily seen.

Neuro-Keratin Network and Axis Cylinder.—Kühne used tryptic digestion for revealing the presence of the neuro-keratin network in the myelin of medullated nerve-fibres. This is not a method capable of being readily employed by the average student. The following method of Platner yields good results:[1]—Place a fresh sciatic nerve—*e.g.*, of a Frog—in a dilute solution of ferric chloride, consisting of—

Liquor ferri perchloride, . .	1 part.
Distilled water or spirit, . . .	3—4 parts.

Leave the nerve in this fluid for three or four days. Wash every trace of the iron salt out of the preparation. This can readily be ascertained by means of the thiocyanate test. Preserve it in alcohol. Place small portions of the nerve in a saturated solution of dinitro-resorcin in 75 per cent. alcohol. This unites with iron in the tissue, and gives a very durable green tint. Alizarin employed in the same way gives a violet colour.

Tease a fragment, dehydrate it in alcohol, and mount it in xylol balsam.

This is an excellent method. The axis cylinder has a green colour, and stands out sharply. It can be traced with ease

[1] *Zeits. f. wissen. Mikros.*, VI. 186.

through several inter-annular segments. The nodes of Ranvier are also sharply defined, while a network of fibres in the myelin, corresponding, according to Platner, to the neuro-keratin network of Kühne, is stained green. The method also succeeds with nerves hardened in Müller's fluid or picro-sulphuric acid.

Transverse Markings on Axis Cylinders and Nerve-Cells.—The existence of alternate dark and clear or lighter brown transverse markings, known as Frommann's lines, on the axial cylinders of cerebrospinal nerves is well known; but, judging from the ordinary text-books, the existence of similar markings on the axis cylinders of the central nervous system, and also on the nerve-cells of the spinal cord, does not seem to be so well known. This is all the more remarkable considering how readily they can be prepared. These striæ are so regular in their arrangement as to remind one at the first glance of the alternate clear and dim discs of striped muscle. Five years after the appearance of Frommann's[1] paper Grandry[2] described a method for demonstrating these lines. He used the spinal cord of the ox and the sciatic nerve of the frog. The organs must be quite fresh, and placed in the silver solution immediately after death. The objects are placed in silver nitrate solution (1 : 400), and kept in the dark, at the ordinary temperature, for four to five days. After this they are exposed to daylight in the same solution for two to three days. Fragments are teased in acidulated glycerine.

The transverse striation on the axis-cylinder is regular, and is composed of alternate dark (brown) and clear (or very faintly brownish) stripes, as if the silver had acted on one part and not on the other; the brown stripes have a certain breadth, which is not quite constant along the course of the fibre, while the distance between the brown bands, *i.e.*, the clear part, may also vary at different parts of the cylinder. The intermediate or clear part is either completely colourless or faintly tinged with brown. Both Frommann and Grandry found that longitudinal striation was occasionally to be observed on the same axis-cylinders on which the transverse markings were distinctly seen.

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[1] "Zur Silberfärbung der Axencylinder," Virch. *Arch.*, XXXI.

[2] "De la structure intime du cylindre de l'axe et des cellules nerveuses," *Journal de l'Anat. et de la Physiol.*, de C. Robin, Paris, 1869. See also Schwann, *Bulletin de l' Acad. des Sciences de Belgique*, 37^e Année, 2^e Série, vol. XXV. p. 287.

On employing the same method for the large multipolar nerve-cells of the anterior cornea of the grey matter of the spinal cord of the ox, only leaving the tissue for a longer time in the staining fluid, Grandry found similar transverse markings on the nerve-cells. The finer striæ appeared homogeneous, but the larger brown ones appeared to be composed of an aggregation of brown or black grains united together. The striæ exist only on the body of the cells and its processes, but they do not occur on the nucleus. Longitudinal striation also exists in the cell. As to the significance of this transverse striation, some observers, such as H. D. Schmidt[1] and R. Arndt,[2] say that they are due to the structural properties of the axis-cylinders; others, *e.g.*, Boveri and Schiefferdecker, regard them as artificial productions.

To reveal these markings, the best method is that of Jakimovitch.[3] The strength of the silver nitrate solution varies from ·25–1 per cent., according to the nature of the tissue. The ·25 per cent. solution is employed for fibres in the central nervous system—·5 per cent. for peripheral nerves and 1 per cent. for nerve-cells. Very small parts of the perfectly fresh tissue are placed in a large quantity of the silver solution and kept in darkness. After twenty-four hours, the fluid being renewed several times in the interval, the pieces are well washed in water and exposed to light until they become brown. The tissue is then placed in the following mixture, and exposed to the light for several days—

Formic acid, . . .	1 part.
Amylic alcohol, . . .	1 part.
Water, . . .	100 parts.

In this fluid the tissue becomes somewhat more transparent, as part of the reduced silver is redissolved by the acid, hence it is necessary to change the solution from time to time. When all the silver that can be dissolved has passed into solution, what remains assumes a brownish tint. For Frommann's lines or axis-cylinder so long a time is not required as for nerve-cells. In the case of the latter, the object is left in silver nitrate for forty-eight hours, and five to seven days in the acid fluid. The preparation is mounted in dilute glycerine.

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[1] "On the construction of the Dark or Double-bordered Nerve-Fibre," *Monthly Mic. Jour.*, XI. p. 200.

[2] "Etwas über die Axen-Cylinder der Nervenfasern," Virch. *Arch.*, vol. LXXVIII.

[3] "Sur la structure du cylindre-axe et des cellules nerveuses," *Jour. de l' Anat. et de la Physiol.*, XXIV., 1888, p. 142.

In order to obtain the striation, the nerve-tissue should be as fresh as possible, preferably from a freshly-killed animal. Grandry gives the limit as six hours after death, after which, according to him, the experiment does not succeed; but, in the case of the spinal cord of the ox, I have succeeded with a cord removed twelve hours after death, but this applies to the axis-cylinders only. In some cases the clear bands are broader than the dark bands, and in other cases the reverse is the case. According to Jakimovitch these markings traverse the whole thickness of the axis-cylinder, and have nothing to do with the axilemma. On this and other grounds he thinks that the axis-cylinder is made up of fibrils consisting of "nervous particles," alternate discs, differing in chemical constitution, and therefore behaving differently to silver nitrate. No such transverse striation, however, is to be obtained with non-medullated nerve-fibres.

To see the transverse striation on the cells, the tissue is placed in a stronger solution of silver and exposed for a longer time to light. So far only the large multipolar nerve-cells of the grey matter of the spinal cord have been shown to exhibit transverse striations by this method.

Dry Preparations of the Spinal Cord.—On a slide stain a transverse section of a spinal cord with methylene blue. Remove the surplus dye and allow the section to dry on a slide. When it is thoroughly dry add a drop of balsam and cover the preparation. There is a sharp differentiation of the individual parts of the section. The cells are shrunk somewhat, but otherwise the appearance of the section is not greatly altered by the process, and the result is a preparation agreeable to the eye.

Golgi's Method for Central Nervous System.[1]—This method only requires to be tried, and satisfactory results are obtained with a little care. It is one which can readily be practised with success by a student. Harden the brain and spinal cord in Müller's fluid or 2 per cent. potassic bichromate for six weeks or longer. Small pieces of the hardened brain are placed in a small quantity of a quarter per cent. silver nitrate, and washed in this until all the surplus silver on the surface of the piece of tissue is transformed into silver chromate. The pieces are afterwards placed for forty-eight hours or longer in ·25 per cent. silver nitrate, and the whole kept in the dark. A half per cent. solution of mercuric chloride may be used instead of the silver, but the latter yields results more speedily. Harden the tissue in alcohol, make moderately thin sections from the surface. The first section is almost certain to contain a large number of black patches of deposited silver, but the next section will yield good results. Care must be taken that the plane of section runs parallel to the direction of the cells and their processes.

In properly prepared sections which are mounted in balsam without a cover-glass being applied one can see the nerve-cells and their finest processes quite black, and one realises the wealth of branched processes possessed by cerebral, cerebellar, and spinal nerve-cells in a way that is not possible by any other method. Sometimes it happens that the silver is largely deposited on the blood-vessels, so that in sections of the spinal cord one can readily trace the distribution of the spinal vessels within the medulla by the blackened deposit which exactly follows the distribution of the blood vessels.

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[1] *Sulla fina anatomia degli organi del sistema nervoso*, Milan, 1876.

A Modified Form of Fat Extraction Apparatus.

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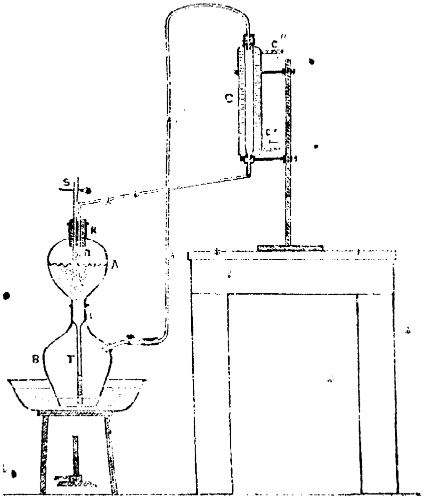
THE fat extraction apparatus described below claims to be nothing more than a convenient modification of one in fairly constant use, viz., Drechsel's. It possesses, however, advantages which perhaps justify a somewhat detailed description.

It consists of two flasks, the lower one (B) possessing a rather narrow base. Thus any fluid in this lower flask will extend, for its bulk, a fair height up the flask. The upper flask (A) fits by a ground glass joint (L) into the neck of the lower, and a more constricted tube extends from the bulbous portion

FIG. 1.



Fat Extraction Apparatus.—A, upper flask; B, lower flask; T, tube opening from lower end of A and reaching almost to bottom of B; C, upright condenser; S, tube closed by burette clip.

forming the stopper as it were, to within a short distance from the bottom of the lower flask. In addition, the lower flask has a tube, fitting also by a ground glass joint, opening from its side in the position seen in the diagram, and provided with a tap.

Into the upper flask is fitted a cork, and through this cork pass two glass tubes, one of which comes from the lower end of the inner chamber of an upright condenser. The second tube passing through the cork (*K*) of the upper flask is closed with a small piece of indiarubber tubing, and this is clipped with an ordinary burette clip. The lower flask is, when in use, placed in a hot bath in the usual manner. I prefer using a large basin, though the loss by evaporation is more rapid, since this is compensated by the change of temperature of the water being very slow; in fact, it is possible to leave the apparatus for a considerable time, if the flame of the Bunsen burner be properly adjusted, without any change of consequence occurring.

It will be unnecessary to do more than point out how the circulation of the ether can only be in one direction, viz., from the tube at the side of the flask (*B*), through the condenser, and then to the upper flask. It is of course blocked from the upper flask by the column of fluid in the tube T. The ether, therefore, should always be put in the lower flask in sufficient quantity (allowing for decrease when part is in circulation), to reach well above the end of this tube.

It will be well, perhaps, to briefly describe the working. The substance from which the fat is to be extracted is placed on the plaited filter in *A*. A sufficient quantity of ether is poured over it, and then passes into the lower flask. The cork at K is replaced, and the apparatus is then fixed (by an arrangement not shown in the diagram), so that the lower part of *B* is well immersed in the water bath. I generally remove the clip at *S* till the ether has got into thorough circulation. It soon commences to pass in a regular stream (not simply a succession of drops), from the tube at *R*. The clip *S* is then replaced, very little ether having been lost by its removal. The pressure in the lower flask can be tested at any time by loosening the clip and observing the height of the column of fluid in *T*. This of course very soon falls, however, after such loosening. The clip furnishes, therefore, a ready means of lowering the pressure.

When thoroughly started, the apparatus requires very little attention. I have left it entirely for two hours, and found on return that it required no re-adjustment beyond the addition of water to the water bath.

When the fats are sufficiently extracted from the substance on the filter, the ether can be recovered in a very simple manner. The cork at *K* is removed from the upper vessel and inserted into a suitable flask. The lower flask is closed at *L* by a stopper fitting it. The ether continues to vaporise, but it is condensed now into the flask instead of passing again into *B*. If the fats are being quantitatively estimated, it is necessary to wash the end of *T*, within and without, with a little fresh ether, and add the same to that in *B*.

The advantages I find, by experience, the apparatus possesses over Drechsel's are the much more rapid circulation of the ether and the greater safety and freedom from likelihood of explosion. It is naturally an advantage to have the ether completely condensed before reaching *A*, instead of filling *A* with a mixture of cold condensed ether and hot ether vapour. In fact, the circulation in the two forms of the apparatus may to some extent be compared to that of the blood in mammals on the one hand and reptiles on the other.

I think, however, that the freedom from probable explosion, which I believe the arrangement obtains, is not its least advantage.

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